Effect of BTN162b2 and CoronaVac Boosters on Cellular Immunity of Individuals Previously Fully Vaccinated with CoronaVac Against SARS-CoV-2: A Longitudinal Study

by

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Koç University

Graduate School of Health Sciences

This is to certify that I have examined this copy of a master's thesis by

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and that any and all revisions required by the final
examining committee have been made.

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Date: June 6, 2022

To the most important two people in

my life…who made me who I am,

who were and will always be with

me in any condition.

ABSTRACT

Effect of BTN162b2 and CoronaVac boosters on cellular immunity of individuals previously fully vaccinated with CoronaVac against SARS-CoV-2: A longitudinal study

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In Turkey, 2 doses of inactivated vaccine (CoronaVac) were first used to protect from COVID-19. Then, to boost immunity, one dose of mRNA vaccine (BioNTech) or one dose of inactivated vaccine (CoronaVac) was administered. This study aimed to evaluate the cellular immunity developed against SARS-CoV-2 after 2 doses of CoronaVac and one dose of BioNTech or CoronaVac boosters. In this study, 33 individuals who are vaccinated with 2 doses of CoronaVac and had no history of Covid-19 were recruited. The study population was divided into 2 groups: Group A, individuals who received 2 doses of CoronaVac and a dose of Pfizer/BioNTech (n=27). Group B, 2 doses of CoronaVac and 1 dose of CoronaVac (n=6). Blood samples were collected 3-5 months after 2 doses of CoronaVac and 1 month after the 3rd dose of either Pfizer/BioNTech or CoronaVac. PBMC isolation was performed by density gradient centrifugation method and IFN-γ and IL-2 cytokine responses were tested by ELISpot after activation with SARS-CoV-2 spike specific peptide mixture. Activated effector helper T cell ($CD4^+CD38^+CD69^+$) and activated effector cytotoxic T cell ($CD8^+CD38^+CD69^+$) responses were investigated by flow cytometry after the induction with SARS-CoV-2 spike specific peptide mixture.

The median of IFN-γ spot/10^6 cells was 60 after 2 doses of CoronaVac and has been increased to 140 in group A in the 1st month ($p<0.0001$) and decreased to 80 in the 3rd month while in group B it was increased to 120 ($p= 0.39$). in the 1st month and decreased to 52 in the 3rd month ($p>0.05$) The median of IL-2 spot/10^6 cells was 20 after 2 doses of CoronaVac and it was 60 in group A ($p<0.0001$) in 1st month and 30 in the 3rd month ($p>0.05$) while 30 spots were calculated in group B in the 1st month ($p= 0.529$) and 25 spots in the 3rd months ($p>0.05$). In the assessment of $CD4^+$ cell and $CD8^+$ cell ratios after stimulation with a mixture of spike peptides, there were no significant changes in the magnitude of $CD4^+$ and $CD8^+$ T cells after BNT162b2 and CV boosters compared to baseline levels. The median ratio of effector $CD8^+CD38^+CD69^+$ T cells and $CD4^+CD38^+CD69^+$ T cells after 2 doses of Coronavac were 1.160 and 4.375 respectively. There was no significant difference compared to baseline levels whereas a significant increase was detected in the 3rd month in effector $CD8^+CD38^+CD69^+$ and $CD4^+CD38^+CD69^+$ T cells.

The magnitude of BNT162b2 booster T cell responses was significantly higher than the CoronaVac booster. Thus, increased IFN-γ and IL-2 reactivity after the BNT162b2 booster, accompanied by an increase in effector $CD8^+CD38^+CD69^+$ T cells were detected. The ratio of effector $CD4^+CD38^+CD69^+$ cells were also high in the 3rd month of the BNT162b booster.

ÖZETÇE

CoronaVac ile SARS-CoV-2'ye karşı tam olarak aşılanmış bireylere uygulanan BTN162b2 ve CoronaVac aşılarının hücresel bağışıklık üzerindeki etkisi: Boylamsal bir çalışma

Rojbin EL

İmmünoloji, Yüksek Lisans

06.06.2022

Türkiye'de ilk kez COVID-19'dan korunmak için 2 doz inaktif aşı (CoronaVac) kullanıldı. Ardından bağışıklığı artırmak için bir doz mRNA aşısı (BioNTech) veya bir doz inaktif aşı (CoronaVac) uygulandı. Bu çalışma, 2 doz CoronaVac ve bir doz BioNTech veya CoronaVac ek doz sonrası SARS-CoV-2' ye karşı geliştirilen hücresel bağışıklığı değerlendirmeyi amaçladı. Bu çalışmaya 2 doz CoronaVac aşısı olan ve Covid-19 öyküsü olmayan 33 kişi alındı. Çalışma popülasyonu 2 gruba ayrıldı: Grup A, 2 doz CoronaVac ve bir doz Pfizer/BioNTech (n=28) olan kişiler. Grup B, 2 doz CoronaVac ve 1 doz CoronaVac (n=6) olan kişiler. Kan örnekleri, 2 doz CoronaVac'tan 3-5 ay sonra ve 3. doz Pfizer/BioNTech veya CoronaVac'tan 1 ay ve 3 ay sonra alındı. PBMC izolasyonu yoğunluk gradyanlı santrifüj yöntemi ile gerçekleştirildi ve SARS-CoV-2 spesifik peptivatör ile aktivasyondan sonra IFN-γ ve IL-2 sitokin yanıtı ELISpot ile yapıldı. Aktif efektör yardımcı T hücreleri ($CD4^+CD38^+CD69^+$) ve aktif efektör sitotoksik T hücresi ($CD8^+CD38^+CD69^+$) yanıtları, SARS-CoV-2'ye özgü peptivatör ile indüksiyondan sonra akım sitometrisi ile araştırıldı.

IFN-γ spot/10^6 hücrelerinin ortancası, 2 doz CoronaVac sonrası 60 iken grup A'da 1. ay 140'a ($p<0.0001$) bulundu ve 3. ay 80'e indi ($p>0.05$). Grup B'de 1. ay 120'ye ($p= 0.39$), 3. ay ise 52.50'ye ($p>0.05$) düştü. 2 doz CoronaVac sonrası IL-2 spot/10^6 hücre ortancası 20 iken grup A'da 1. ay 60 ($p<0.0001$), 3. ay 30 ($p>0.05$) iken grup B'de 1. ay 30 ($p= 0.529$), 3. ay 25 spot teshis edildi ($P>0.05$).

Spike peptit karışımı ile stimülasyondan sonra $CD4^+$ hücre ve $CD8^+$ hücre oranlarının değerlendirilmesinde, BNT162b2 ve CV ek dozlarından sonra $CD4^+$ ve $CD8^+$ T hücrelerinin sayılarında bazal düzeye kıyasla önemli bir değişiklik olmadı. 2 doz Coronavac sonrasında efektör $CD8^+CD38^+CD69^+$ T hücrelerinin ve $CD4^+CD38^+CD69^+$ T hücrelerinin medyan değeri sırasıyla 1.160 ve 4.375 idi. Bu hücre düzeylerinde başlangıç seviyelerine göre anlamlı bir fark bulunmazken üçüncü ayda anlamlı bir artış tespit edildi.

BNT162b2 ek doz sonrası güçlendirici yanıtı, CoronaVac ek dozundan daha yüksekti. BNT162b2 sonrası artan IFN-γ ve IL-2 reaktivitesine efektör $CD8^+CD38^+CD69^+$ T hücrelerindeki artış da eşlik etti. BNT162b ek doz sonrası 3. ayda da efektör $CD4^+CD38^+CD69^+$ hücre oranında artış gözlemlendi.

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α	alpha
APC-Cy7	Allophycocyanin-cyanine7
BCoV	Bovine Coronavirus
BNT162b2	BioNTech
BSA	Bovine Serum Albumin
BV510	Brilliant violet 510
C	Celcius
CD	Cluster of Differentiation
$CD4^+$	Helper Cell
$CD8^+$	Cytotoxic Cell
$CD38^+$	Effector Cell
$CD69^+$	Activated Cell
$CD45RA^+$	Naiive Cell
$CD45RO^+$	Memory Cell
CMV	Cytomegalovirus
CoV	Coronaviruses
COVID-19	Coronavirus Disease -2019
cRPMI	Complete RPMI
CV	CoronaVac
Ct	Cycle threshold
CXCL13	Chemokine (C-X-C Motif) ligand 13
DMSO	Dimethyl sulfoxide
DPBS	Dulbecco's phosphate-buffered saline
E	Envelope Protein
EDTA	Ethylendiamintetraacetat
ELISA	Enzyme-linked Immunoassay
ELISpot	Enzyme-linked immune absorbent spot
EtOH	Ethanol
FACS	Fluorescent Activated Cell Sorting
FBS	Fetal Bovin Serum
FCS	Fecal Calf Serum
FITC	Fluorescein Isothiocyanate
H	Hour
HCW	Health Care Workers
HLA	Human Leukocyte Antigens

IFN	Interferon
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
IL-2	Interleukin-2
IFN- γ	Interferon-gamma
M	Membrane Protein
MERS	Middle East Respiratory Syndrome
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
MHV	Mouse Hepatitis Coronavirus
MPC-1	Mitochondrial Pyruvate Carrier
N	Nucleoprotein
PHA	Phytohemagglutinin
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate Buffered Saline
PMA	Phorbol 12-myristat 13-acetat
PE-Cy7	Phycoerythrin cyanine 7
PE-Dazzle 594	Phycoerythrin Dazzle 594
S	Spike Protein
SARS	Severe Acute Respiratory Syndrome
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus-
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus-2
TCR	T cell Receptor
TCM	Central Memory T cells
TEM	Effector Memory T cell
Th	T helper
Tc	T Cytotoxic
TNF	Tumor Necrosis Factor
UV	Ultraviolet Light
WHO	World Health Organization

CHAPTER 1:
INTRODUCTION

SARS-CoV-2 is a virus that was first detected in the city of Wuhan, China and causes respiratory tract infection; It causes COVID-19. This disease manifests itself with symptoms such as high fever, cough, shortness of breath, headache, sore throat, runny nose, muscle and joint pain, weakness, loss of sense of smell and taste, and diarrhea (Ozma et al., 2020).

Coronaviruses (CoV) are a large family of viruses with single-stranded RNA, enveloped, with protein protrusions on the surface. In addition to mild and moderate respiratory diseases, it is also responsible for severe diseases such as MERS (Middle East Respiratory Syndrome), SARS (Severe Acute Respiratory Syndrome), and COVID-19 (Liu et al.,2021).

The agents that provide protection and immunity against SARS-CoV-2 infections are vaccines. Helper memory T cells, cytotoxic T cells, memory B cells, and neutralizing antibodies help determine protection against re-infection, disease risk, and vaccine efficacy. However, the relationship between the cellular immune memory in humans, as opposed to SARS-CoV-2, is not fully known (Braun et al., 2020).

In the following sections, cellular immunity developed against SARS-CoV-2 vaccination, and the possible booster doses are introduced.

1.1 SARS-CoV-2 Taxonomy

Coronaviruses are members of the Coronaviridae family of the Nidovirales order. Coronaviruses have been divided into four genera: α-, β-, γ-, and δ and - coronaviruses (Wu et al.,2020). - and - CoVs infect mammals, - coronaviruses infect birds, and - coronaviruses infect both mammals and birds. - coronaviruses include SARS-CoV, mouse hepatitis coronavirus (MHV), MERS-CoV, bovine coronavirus (BCoV), bat coronavirus HKU4, and human coronavirus OC43, which includes SARS-CoV-2 (Figure 1.1) (Fehr et al., 2015). All three CoVs, SARS-, MERS-, and SARS-CoV-2, are spread among humans by zoonotic transmission and intimate contact. The primary reproduction number (R0) of SARS-CoV-2 person-to-person dissemination is around 2.6, implying that infected cases multiply exponentially (Hellewell et al., 2020).

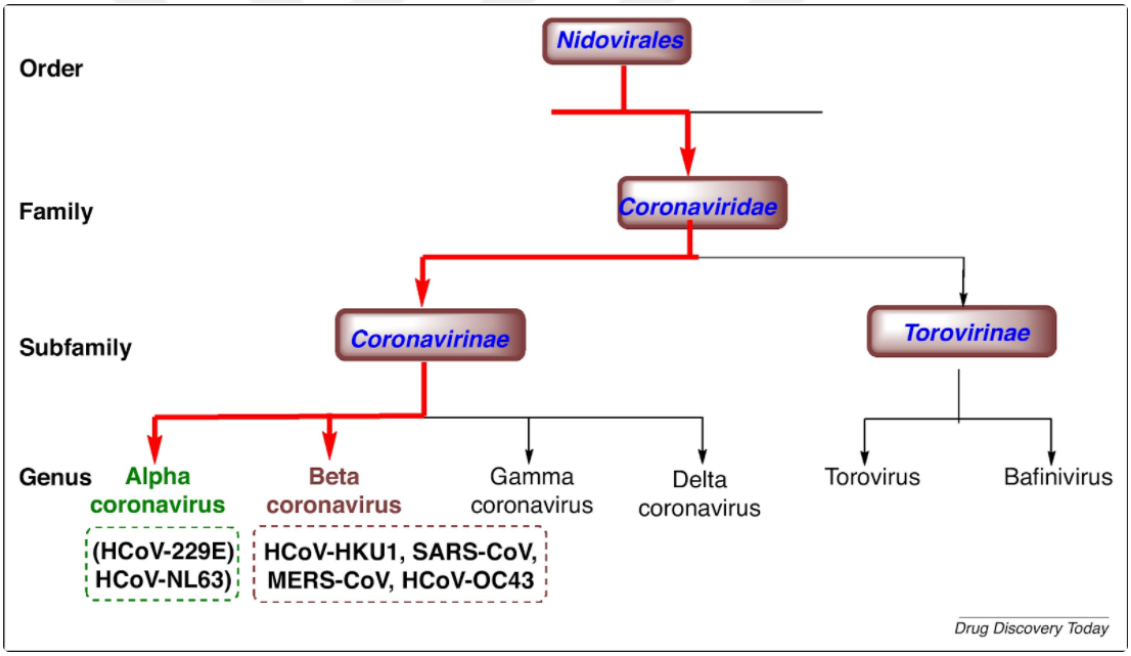


Figure 1.1: The taxonomy of the Coronaviridae family of viruses according to ICTV classification, with coronaviruses being known to infect humans highlighted (Pillaiyar et al., 2020).

1.2 General Properties of SARS-CoV-2 Structure

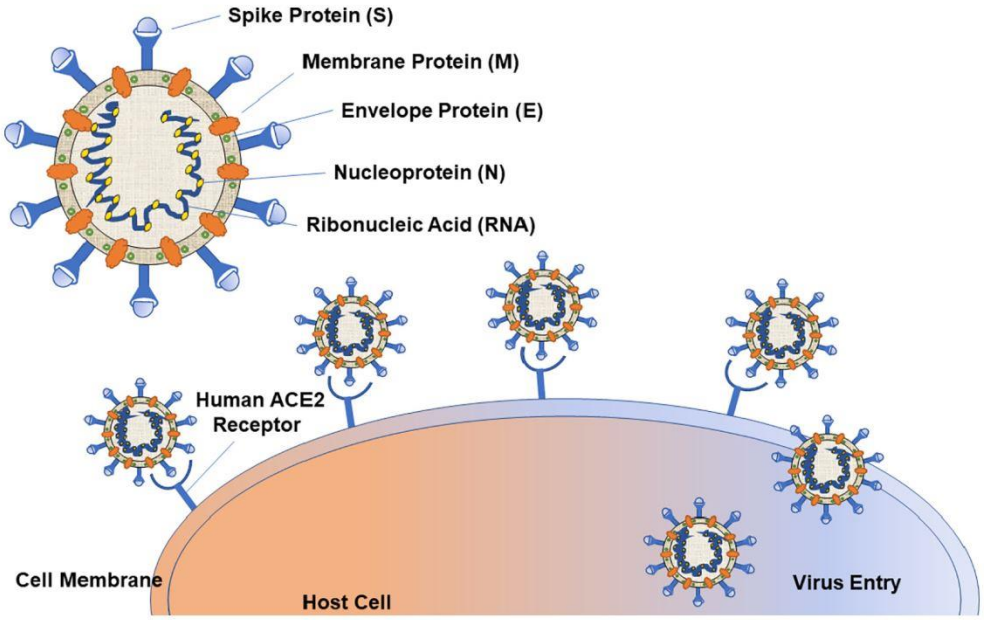


Figure 1.2: The SARS-CoV-2 structure, and method of host entrance are depicted schematically (Naqvi et. al, 2020).

CoVs have the biggest RNA viral genomes, with genome sizes ranging from 26 to 32 kb (Huang et al, 2020). SARS-CoV-2 shares roughly 82 percent sequence identity with SARS-CoV and MERS-CoV, and > 90 percent sequence identity for key enzymes and structural proteins with SARS-CoV and MERS-CoV. Because of the high level of the sequence, a common pathogenic mechanism was discovered, allowing for therapeutic targeting. Spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins are among the four structural proteins found in SARS-CoV-2 (Figure 1.2). These proteins have a high degree of sequence similarity to the SARS-CoV and MERS-CoV equivalent proteins.

1.3 SARS-CoV-2 Infection

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epidemic emerged in Wuhan, China in December 2019 and spread rapidly all over the world. On January 30, 2020, Covid-19 disease was declared a pandemic by the human-to-human transmission and the World Health Organization Emergency Committee. Currently, Covid-19 is recognized as a high-risk infectious agent all over the world, with an overall

mortality rate of 0.5% to 3.5% (Gómez,2020). While some of the patients have a severe illness with symptoms similar to a number of respiratory tract infections and pneumonia, some patients remain asymptomatic to infection. (Figure 1.3). Therefore, it is very important to determine the long-term immunological memory of patients with SARS-CoV-2 infection. Helper memory T cells, cytotoxic T cells, memory B cells, and neutralizing antibodies help determine protection against re-infection, disease risk, and vaccine efficacy. However, the relationship between the immune memory type in humans and SARS-CoV-2 is not fully known. (Braun et al., 2020).

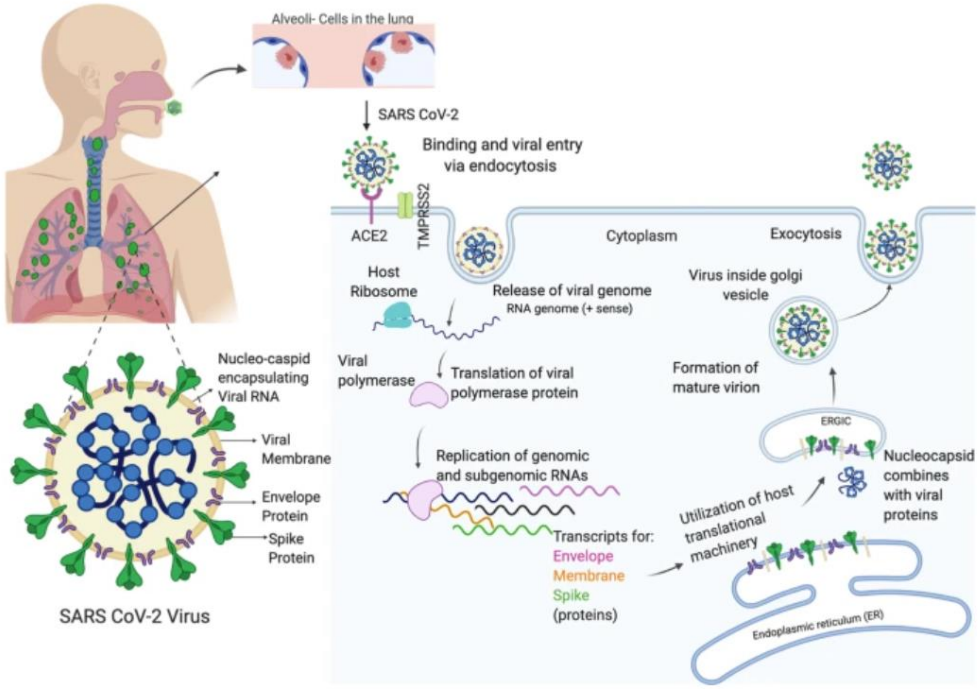


Figure 1.3: Molecular drivers of SARS-CoV-2 productive infection (Chakravarty et al., 2020).

1.4 Viral Replication of SARS-CoV-2

SARS-CoV-2 uses the human ACE-2 receptor as an entry receptor into host cells and human proteases as entry activators (Mahmoud et. al., 2020). Spike protein mediates entry of SARS-CoV-2 into cells and binds to ACE-2 receptors via its receptor binding site (RBD) (Lima et. al., 2021). After Sars-CoV-2 enters human host cells, viral RNAs

are released and Toll-like receptors (TLR3, TLR7, and TLR9) are induced by pattern receptors (PRRs). Signaling at these cell receptors induces cytosolic translocation of various nuclear transcription factors such as NF-kB and activating protein (AP-1) to the cell nucleus, transcription, and expression of genes. Acute inflammatory response proteins such as C-reactive protein (CRP), proinflammatory cytokines, and chemokines (such as IL-1, IL-6, TNF-α, MPC-1, IL-8, and IP-10) and interferons acting in viral control (IFNs) trigger the antiviral state (Figure 1.4). (Kawai et. al., 2006). Accumulating evidence suggests that the severity of COVID-19 is associated with increased levels of inflammatory mediators, including cytokines and chemokines (Kempuraj et. al., 2020).Therefore, it is important to examine the cytokine responses of memory T cells in vaccinated groups in this study.

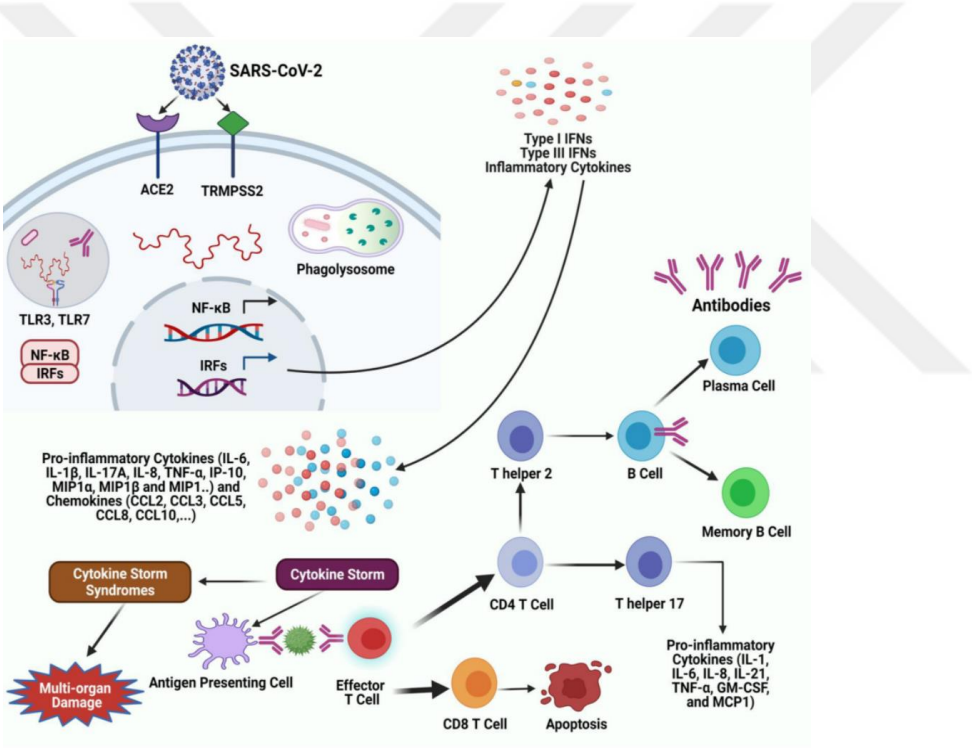


Figure 1.4: Immune responses to SARS-CoV-2 (Rabaan et. al., 2021).

1.5 T cell response to SARS-CoV-2

The adaptive immune response, which underlies vaccine efficacy, is a critical driver of clinical outcomes after SARS-CoV-2 infection. T cell responses emerge early and are associated with protection, but they are hampered in severe illness and are linked to high activation and lymphopenia (Shrotri et. al., 2021). SARS-CoV-2 cross-reacts with

a fraction of T cells sensitized against seasonal coronaviruses, which may contribute to clinical protection, especially in the early stages of life (Moss et. al., 2022). T cell memory encompasses broad recognition of viral proteins, believed to be around 30 epitopes per person, and appears to be stable so far (Saini et. al., 2022). This broad recognition can reduce the impact of particular viral alterations and is believed to support protection against severe diseases caused by viral variations. Current COVID-19 vaccinations evoke strong T-cell responses, which are thought to play a role in the vaccine's extraordinary protection against hospitalization or death, and innovative or heterologous regimens have the potential to improve cellular responses even more. T cell immunity plays a critical role in the regulation of SARS-CoV-2, and its significance may have been neglected thus far (Figure 1.5.) (Grifoni,2020).

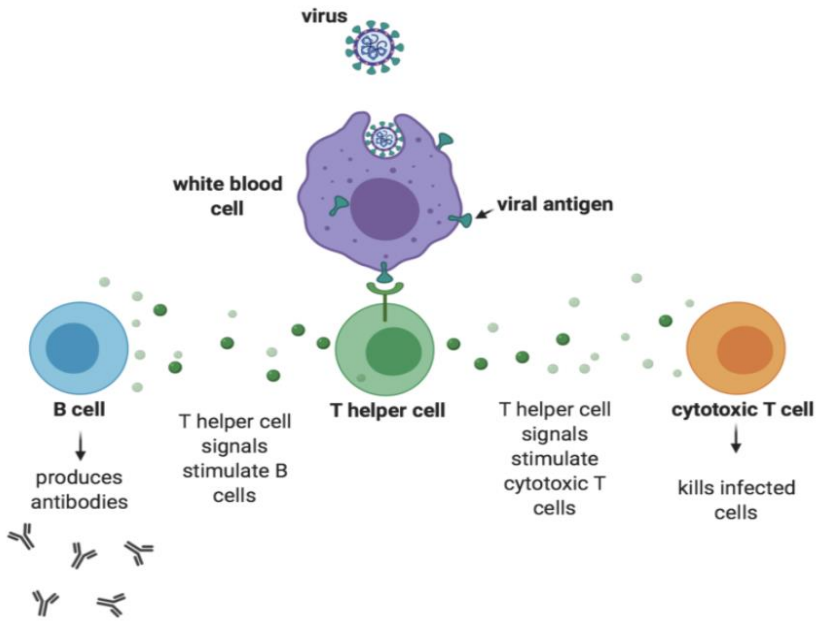


Figure 1.5: T cell Response against SARS-CoV-2 Illustration (Gertrud, 2020).

Memory T cells are a subgroup of T lymphocytes, which are active cells of adaptive immunity. T cells that encounter antigen-presenting cells are stimulated by immunological synapse with $CD28^{+}$ and CD49d and HLA glycoproteins loaded with antigen and markers on the surface of the antigen-presenting cell. As a result of stimulation, IL-2 proliferates and polarizes, thus creating an immune response (Chang et. al., 2014 and Song et. al., 2005). When the infection ends, active T cells are suppressed with the support of helper memory T cells, and intracellular inflammation

ends. However, some T cells with $CD45^+$ markers on their surface transform into $CD45RO^+$ antigen by changing their receptors (M Opata et. al., 2013) With this transformation, T cells are transformed into memory T cells. Memory T cells accelerate the TCR signaling pathway with the $CD45RO^+$ antigen receptor, increasing the speed of the cell's rapid immune response, thus a rapid reaction against the infection occurs (Kaech et. al., 2002 and Mahnke et. al., 2013). As mentioned above, Memory T cells are also divided into two as auxiliary memory and cytotoxic memory T cells. By flow cytometry immunophenotyping, helper memory T cells are defined as $CD45RO^+CD3^+CD4^+$ and cytotoxic memory T cells are defined as $CD45RO^+CD3^+CD8^+$. Later, these cells are divided into two central memory T cells (TCM) and effector memory T cells (TEM). Central memory T cells have the $CCR7^+$ receptor, whereas effector memory T cells do not express $CCR7^-$ (Figure 1.6) (Benichou et. al., 2017). Central memory T cells are found in lymph nodes, but upon a second encounter with the same antigen, they proliferate and become effector memory T cells. Effector memory T cells are commonly found in peripheral blood, and when intracellular infection occurs, they generate cytokine responses by producing IFN-γ and TNF-alpha (Kallies et. al., 2008)

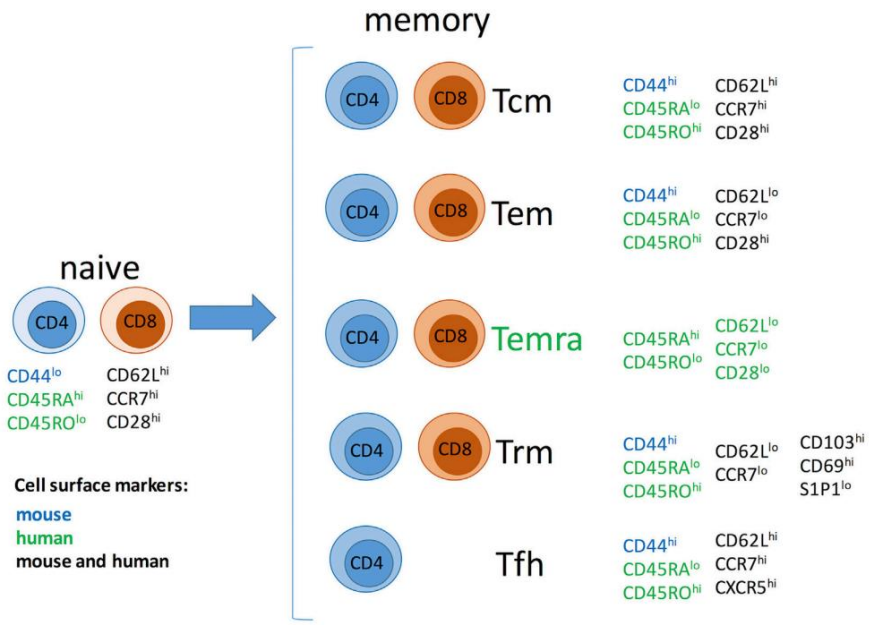


Figure 1.6: Memory T cell subsets. Abbreviations: Tcm, central memory T cells; Tem, effector memory T cells; Temra, terminally differentiated effector memory T cells; Trm, resident memory T cells; Tfh, follicular helper memory T cells (Benichou et. al., 2017).

1.6 SARS-CoV-2 Vaccines

The agents that provide protection and immunity against SARS-CoV-2 infections are vaccines. 182 different vaccine studies are ongoing all over the world and only 11 vaccines have been approved by the FDA (Strizova et. al., 2021) Countries around the world have started to vaccinate their citizens in line with their vaccination policy. The vaccines used vary from country to country. Mostly used vaccines in Europe and America are those using encapsulated mRNA technology (Hussain et. al., 2022). In our country, the vaccine produced with inactivated virus technology was first opened to all healthcare workers and the elderly population, and then to the entire population. In addition, some volunteers, especially healthcare workers, participated in the phase studies of the vaccine using encapsulated mRNA technology. Currently, vaccines produced with mRNA technology are also routinely administered to citizens in our country. The protection of the currently administered inactivated vaccine in our country has been reported as 91.25%. (19 vaccine production technologies,2022). It has been shown that the protection of the vaccines produced using encapsulated mRNA technology in phase 3 study results varies between 94% and 97% (Pardi et. al., 2018).

The literature contains various information about the protection of vaccines alone, but the immune efficacy of vaccines produced with different technologies has not been compared in studies so far. The fact that these two types of vaccines have been applied to different groups in Turkey provides a basis for comparing the efficacy of these vaccines with each other. In this project, it is planned to examine cytotoxic activated effector T cell ($CD8^+$), and helper activated effector T cell ($CD4^+$) on blood samples taken at 1^{st} and 3^{rd} months from volunteers who had inactivated virus and encapsulated mRNA vaccines. Helper memory T cells both assist in antibody production and play a role in activating memory B and cytotoxic T cells (Alberts et. al., 2002). Antigen-specific IgG antibodies produced by memory B cells have neutralization properties(Lederer et. al., 2020). If the same antigen is encountered again, the IgG antibody produced by the memory B cells ensures the neutralization of the virus. The aforementioned cells differentiate from $CD45RA^+$ multipotent hematopoietic stem cells to myeloid and lymphoid precursor cells. According to different cytokine responses, these cells differentiate into T, B, dendritic, and natural killer cells (Spits et. al., 1995 and Freud et. al., 2006).

According to a study done in China, for the CoronaVac vaccine they have found that adaptive immune responses especially $CD4^+$ and $CD8^+$ T cell gave response to the inactivated vaccine. $CD4^+$ T cells and $CD8^+$ T cells specific for SARS-CoV-2 were linked to a lower disease severity (Grifoni et al.,2020 and Moderbacher et. al., 2020). Moreover, they have found out that memory $CD4^+$ and $CD8^+$ T cells biased in favor of Temra and Tem cells (Figure 1.6) (Chen et. al., 2022).These favorable phenotypes were thought to be cytotoxic and long-lived, with the ability to respond quickly to infected cells and eradicate them (Tian et. al., 2017 and Tian et. al., 2019).

Similarly, according to a study done in Budapest, they have detected The BNT162b2 cohort (median 209.4 SFU, group 1, at day 28) had a greater T cell response than the BBIBP-CorV (inactivated vaccine) vaccinated participants (median 103.9 SFU, group 1, at day 42, $p < 0.01$) (Vályi-Nagy et. al., 2021). Furthermore, they revealed that In BBIBP-CorV vaccinated healthy volunteers and convalescent patients, the magnitude and specificity of T cell responses to SARS-CoV-2 structural proteins (S, N, and M)

were similar. The mRNA-based BNT162b2 vaccination, on the other hand, was able to elicit a substantially larger T cell response to the spike protein (S1 and S2 peptide pools), but no anti-nucleocapsid or anti-membrane protein-specific T cell response (Vályi-Nagy et. al., 2021).

In general, the mRNA vaccine (BNT162b2) induces T cell responses that narrowly target the spike protein most prone to alterations (Figure 1.7) whereas the inactivated viral vaccine (Figure 1.8) induces T cell responses that target epitopes of spike, nucleocapsid, and membrane proteins.. Therefore, it is possible that inactivated vaccine (CoronaVac) which targets several epitopes could reduce the risk of immunological escape due to new mutations compared to mRNA vaccines.

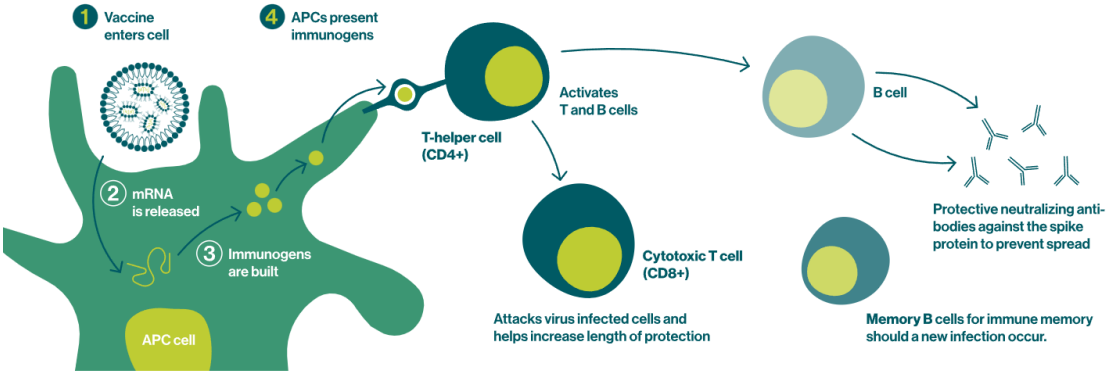


Figure 1.8: mRNA Vaccine Mechanism of Action (Retrieved from BioNTech Home, 2022)

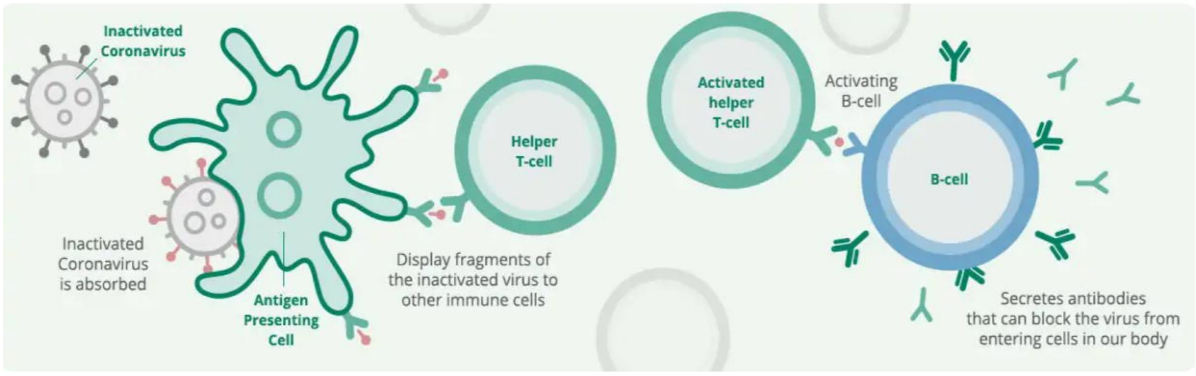


Figure 1.7: Inactivated Vaccine Mechanism of Action (Retrieved from Covid-19 vaccines - sinopharm, 2021).

Within the scope of the presented study, it was aimed to compare the effector T cell responses and effects of the vaccines produced with inactive virus and mRNA technologies applied in our country at 1 and 3 months in individuals. Thus, the responses of these two different vaccines we compared for the first time in the world, and it is planned to contribute to the literature. The long-term effects of vaccines administered in our country on the immunity of individuals are unknown. Within the scope of this study, blood was drawn from vaccinated volunteers and healthy donors who have never had the disease, and mononuclear cells have been isolated. Immunophenotyping of Memory $CD4^+$ and $CD8^+$ T cells was performed from these cells. The effector functions of $CD4^+$ and $CD8^+$ T cells against SARS-CoV-2 were examined by evaluation of cytokine responses.

CHAPTER 2:
METHODOLOGY

This study investigated the effect of BTN162b2 and CoronaVac boosters on the cellular immunity of individuals previously fully vaccinated with CoronaVac against SARS-CoV-2. Time-dependent decreases can be seen in the T cell responses originating from vaccines produced with two different vaccine technologies applied in our country. Therefore, additional booster doses may be needed.

Characterization of T cells from peripheral blood mononuclear cells has been done via flow cytometry (Attune NxT Flow Cytometer). Peripheral blood mononuclear cells are stimulated using synthetic SARS-CoV-2 spike peptide mixtures (PepTivator®SARS-CoV-2 Prot S-research grade (6 nmol/peptide, Miltenyi Biotec, Germany). The IFN-γ and IL-2 responses of T cells were measured with Fluorospot Assay (Abcam Fluorospot, USA) according to the manufacturer's instructions.

Informed consent was obtained from all participants. This study was approved by The Institutional Review Board of Koç University under the number 2021. 151.IRB1.055

2.1 Study Design and Participants

The study was conducted in three centers (Koç University Hospital, Istanbul University Cerrahpasa Hospital, and Istanbul University, Istanbul Medical School Hospital) in Istanbul, Turkey. Individuals who had been previously immunized with two doses of CV and had no history of COVID-19 were included in the study. The baseline blood samples were collected three to five months after 2 doses of CV. Follow-up blood samples were taken after the first and third months of the third dose of CV or one dose of the BNT162b2 booster. Participants were continuously monitored for SARS-CoV-2 infection (Kuloglu & El et al., 2022).

In this study, 33 individuals who are vaccinated with 2 doses of CoronaVac and had no history of Covid-19 were recruited. The study population was divided into 2 groups: Group A, individuals who received 2 doses of CoronaVac and a dose of Pfizer/BioNTech (n=27). Group B, 2 doses of CoronaVac and 1 dose of CoronaVac (n=6). Blood samples were collected 3-5 months after 2 doses of CoronaVac and 1 month after the 3rd dose of either Pfizer/BioNTech or CoronaVac. Due to a change in vaccine recommendations based on the variant of concerns, some participants who have received the 4th dose of BNT162b2 or CV vaccine, therefore, were excluded from the study. Consequently, 6 participants from the BNT162b2 booster group and 4 participants from the CoronaVac booster group were able to donate blood samples 3 months after the booster dose (Figure 2.1). (Kuloglu & El et al., 2022).

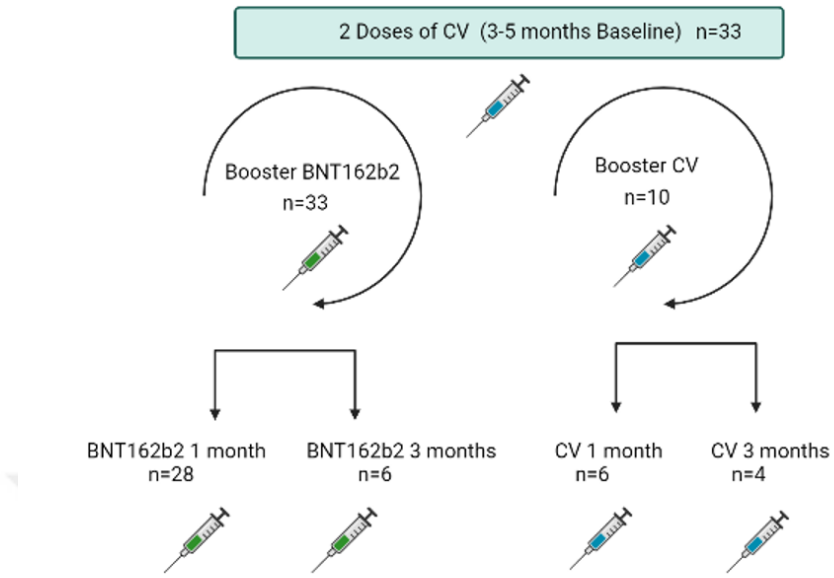


Figure 2.1: The Design of the Study (Created in Biorender, 2022)

The study had some criteria for selection. Inclusion criteria for the study was not having had COVID-19 prior to vaccination which was confirmed by anti-spike antibody ELISA testing, being vaccinated with mRNA or inactivated vaccine, and being in the 18-50 age group. Exclusion criteria for the study were receiving a clinical diagnosis that suppresses the immune response such as immunodeficiency, diabetes, chronic kidney disease, cancer, chemotherapy, SARS-CoV-2 antibody-positive before vaccination, diagnosed with COVID-19 infection during the study (PCR or CT positivity) and COVID-19 symptoms and PCR test positivity during weekly symptomatic follow-up after vaccination. Before vaccination, blood SARS-CoV-2 antibodies in the whole group were investigated by ELISA, and it was confirmed that the volunteers are not infected. During the study, all volunteers were followed for symptoms of COVID-19, and infection was investigated by PCR testing in suspected volunteers (Kuloglu & El et al., 2022).

2.2 Separation of Peripheral Mononuclear Cells (PBMC) from samples by density gradient centrifugation method

PBMCs were isolated from heparinized whole blood by density-gradient sedimentation using Ficoll-Paque according to the manufacturer's instructions (Stem Cell Technology, 07801). The blood samples in green-capped tubes were combined with the aid of a pipette in a 50 ml falcon and diluted 1:1 with PBS 1X (Sigma -Aldrich, USA) and mixed well by pipetting. Lymphoprep (Stem Cell Technology, 07801) was added to another 50 ml falcon at a 1:1 ratio with diluted blood. Lymphoprep-infused falcon is tilted at 45 degrees and the diluted blood is spread slowly with a serological pipette. It was operated with brakeless centrifugation at 2000 rpm, 20 °C, 15 minutes, acceleration 5, and deceleration off.

Buffy Coat part was taken with a pipette and transferred to a 15 mL falcon tube. 1X PBS was added to the obtained buffy coat at a ratio of 1:1 and braked at 2000 rpm, 20 °C, acc: max, dec max. The centrifuge was run for 5 minutes. The supernatant was aspirated or removed with a pipette. Tapping the remaining pellet and adding 1 mL of freezing medium dropwise and pipetting slowly. 10% DMSO (Sigma, D2650-100ml)) + 90% FBS (Gibco, 10500064) to prepare the freezing medium. The prepared mediums were drawn with a syringe, filtered with a 0.22 filter, and kept on ice. The obtained cells were added to cryovial tubes in 1 mL each on ice. It is kept overnight in Styrofoam at -80, then a day later put into boxes and stored at -80°C.

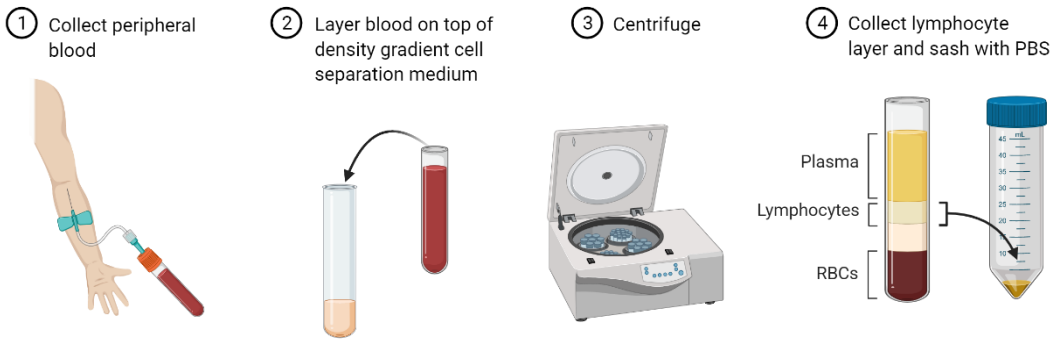


Figure 2.2: Peripheral Blood Mononuclear Cell Isolation Steps (Retrieved from Biorender, 2022).

2.3 Effector CD4⁺ and CD8⁺ Isolation

Mojosort Buffer in default was 5X but it was used as 1X Mojosort buffer according to the manufacturer's instructions. (MojoSort™ Human $CD4^+$ and $CD8^+$ T Cell Isolation Kit, Biolegend, California). Falcon tube containing 1 ml of complete RPMI-1640 medium (Sigma, R8758) was taken and 2 ml of 1X mojo sort buffer were added for both healthy and unhealthy depending on group numbers. To prevent clump formation and bubbling, it was passed from the blue flow strainer tube and centrifuged at 500g for 5 minutes and the flow tubes with pen-strep were removed. Supernatant has been discarded and 100 ul of the medium on each flow tube added.100 ul of 1X Mojosort buffer was added to each tube in total. 200 ul was divided in two flow tubes into four flow tubes as described in the figure and 100 ul for each was added. For $CD4^+$ T cells add 10 ul of biotin antibody and for $CD8^+$ cells were added again add 10 ul of biotin antibody. It was vortexed and kept on ice and incubated on ice for 15 minutes. 2 ml of Mojosort Buffer and centrifuged at 500 g for 5 minutes were added. The supernatant was discarded, and the cells were dissolved with the remaining medium. 10 ul of nanobeads were added to each tube specific for $CD4^+$ and $CD8^+$ and incubated on ice for 15 minutes. 2 ml of Mojosort buffer was added and centrifuged at 500 g for 5 minutes. The supernatant was discarded, and 2.5 mL of mojo sort buffer was added to the first flow tube and put into the magnet and waited for 5 minutes. After 5 minutes, cells were poured the tube into the new tube and for the rest, 1 mL of freezing medium was added and stored at -80 degrees. The same procedure has been done for the other flow tubes as well. For the clear memory T cells, it was centrifuged at 500g for 5 minutes and discarded the supernatant. 10 ul of 2 ng/ ul of IL-2, 2 ul of PHA were added for each flow tube, and 990 ul of complete warm RPMI. However, according to the manufacturer's instructions, the best results were obtained with PBMC. Therefore, Experiments carried out with PBMC isolation rather than T cell isolation.

2.4 Staining Buffer Preparation

Staining buffer was prepared with a mixture of 1X PBS (Sigma -Aldrich, USA) and 1% BSA (5g), filtered, and stored at 4 °C. 500 mL of staining buffer was prepared and aliquoted in 50 mL falcon tubes.

2.5 Antibodies used for the Flow Cytometry Assay

The frequency and characteristics of $CD4^+$ and $CD8^+$ T cells that produce cytokines in peripheral blood measured in vitro using flow cytometry. Peripheral blood mononuclear cells (PBMCs) stimulated with the mitogens which is phorbol. Myristate acetate (PMA) and ionomycin. The duration of stimulation is 5 hours. A chemical blocking the release of proteins from the Golgi complex (Brefeldin A) is added to enhance intracytoplasmic accumulation of cytokines during cell stimulation. In measuring antigen-specific cytokine production, cells treated with ligands for co-stimulatory molecules which is CD28 in addition to antigens. Stimulated cells are fixed and permeabilized for intracellular cytokine staining. Different types of fixations and permeabilization agents are available. Paraformaldehyde (4%) is used as fixation and permeabilizing reagents, respectively. In conjunction with cytokine analysis, cellular characteristics of cytokine producing, and non-producing T cells simultaneously analyzed using antibodies to cell surface and intracellular molecules. The early activation marker CD69 identified by staining cells with antibodies to CD69. Overall, flow cytometric analysis of activated T cells can be a powerful tool in analyzing T cells producing cytokines in health and disease. Below, antibodies used in our study are found (Table 2.1.).

Table 2.1 Antibodies used in the study.

Specificity	Clone	Species	Isotype	Conjugate	Catalog	Company
CD3	SK7	Mouse anti-Human	IgG1, κ	FITC	981002	BioLegend
CD8a	HIT8a	Mouse anti-Human	IgG1, κ	BV510	300934	BioLegend
CD4	OKT4	Mouse anti-Human	IgG2b, κ	PE-Dazzle 594	317448	BioLegend
CD28	CD28.2	Mouse anti-Human	IgG1, κ	Alexa Fluor 700	302920	BioLegend
CD38	HB-7	Mouse anti-Human	IgG1, κ	PE-Cy7	356608	BioLegend
CD69	FN50	Mouse anti-Human	IgG1, κ	APC	310910	BioLegend
CD19	63D3	Mouse anti-Human	IgG1, κ	APC-Cy7	343514	BioLegend
CD20	MOPC-21	Mouse anti-Human	IgG1, κ	APC-Cy7	302218	BioLegend
IFN-y	B27	Mouse anti-Human	IgG1, κ	PE	506507	BioLegend
IL-2	MQ1-17H12	Mouse anti-Human	IgG2a, κ	BV605	500332	BioLegend
TNF alpha	Mab11	Mouse anti-Human	IgG1, κ	BV711	502940	BioLegend

2.6 T cell Flow Assay

For T cell assays, PBMCs are thawed in a 37-degree water bath. 3 mL of RPMI-1640 medium (Sigma, R8758) was taken with 5% AB serum into a 15 ml falcon tube, and PMBC was added dropwise. Centrifugation was performed at 500g for 5 minutes and RBC lysis buffer (Invitrogen, 00-4333) was performed for optimal lysis of erythrocytes. 2 ml of 1X lysate was added and cells were resuspended. Then, the cells vortexed and incubated for 5 min. After incubation, centrifugation was performed at 500g for 10 min.

The supernatant was removed. Pellet was resuspended in an aforementioned buffer. The cell pellet was diluted with 1 ml warm RPMI-1640 Medium (Sigma, R8758) and 50 ul of cells were transferred to the PCR tube for cell counting. Cells were put into the strainer to prevent clump formation and transferred into a new falcon tube. After cell counting, 2 x 10^6 PBMCs were seeded, and all volumes completed up to 200 ul with RPMI-1640 medium (Sigma, R8758) supplemented with 5% AB serum. T cells were activated by using a SARS-CoV-2 Spike protein-specific peptivator (PepTivator®SARS-CoV-2 Prot S-research grade, 6 nmol/peptide, Miltenyi Biotec, Germany) and were incubated for 5 hours at 37°C, 5% CO2. After 2 hours of incubation, 1 ul of Brefeldin A (Biolegend, 420601) was added to the unstimulated and SARS-CoV-2 specific peptivator group to enhance intracellular cytokine staining signals by blocking the transport process during cell activation. After 5 hours of incubation, cells were centrifuged at 500g for 5 minutes. The supernatant was discarded. Cells were washed with PBS buffer (Sigma Aldrich, USA) Zombie NIR dye (Biolegend, 423105) was diluted at a ratio of 1:500 in PBS (Sigma Aldrich, USA). Cells were resuspended in the mixture and 100 ul of solution were spread into each flow tube. The cells were incubated at room temperature in the dark for 15 minutes. After then, cells were washed with staining buffer. The antibody staining procedure was performed as desired. T cell surface marker antibodies CD3-FITC (Biolegend, 344804), CD4-PerCP-Cy5.5 (Biolegend, 367108), CD8-Brilliant Violet 510 (877-246-5343) (Biolegend, 344732), CD38-PE-Cy7 (877-246-5343), (Biolegend, 303516), CD69-APC (KLON FN50), (Biolegend, 310910), CD14-APC-Cy7 (Biolegend, 367108), CD19-APCCy7 (Biolegend, 877-246-5343) were used.

Mixture of fluorochrome cocktail was done in a PCR tube with a dilution of CD3 (1:100), CD4 (1:100), CD8 (1:100), CD38 (1:50), CD69 (1:50), CD14 (1:100), CD19 (1:100). After the mixture, 7 ul antibody cocktails were dispersed to each well from this mixture and incubated at dark for 15 minutes at room temperature. After then, samples were transferred to flow tubes with the Pasteur pipette and centrifuged at 500g for 5 minutes. The supernatant was discarded. 150 ul of Cyto-Fast™ Fix Buffer (Biolegend, 426803) was added for fixation. Incubation was done for 20 minutes at dark at RT. 200 ul of Cyto-Fast™ Permeabilization Buffer 1X was added and centrifuged at 500g for 5 min. Corresponding amount of antibodies with a dilution of TNF alpha Brilliant Violet Mouse anti-Human (Biolegend,502940), IFN gamma PE Mouse anti-Human (Biolegend, 506507), IL-2 Brilliant Violet 605 (Biolegend, 200332) Mixture of fluorochrome cocktail

was done in a PCR tube with a dilution of TNF alpha (1:50), IFN gamma (1:400) and IL-2 (1:50) prepared in 1X Cyto-Fast™ Perm Wash Solution and dispersed to all wells and incubated for 30 min at dark at room temperature. Cell was washed with 1 ml of 1x Cyto-Fast™ Perm Wash solution and centrifuged at 500g for 5 minutes. Supernatants were discarded and cells were washed with Cell Staining Buffer and resuspended in 1 ml of staining buffer.

PMA/Ionomycin (Biolegend, 423302) was used as positive control while CMV (PepTivator® CMV IE-1 –premium grade (6nmol/peptide, Miltenyi Biotec 130-093-493, Germany) was used for cross-reaction, and finally DMSO (Sigma, D2650-100ml) were used as a negative control. Samples were run using Attune Flow cytometer (Attune NxT Flow Cytometer).

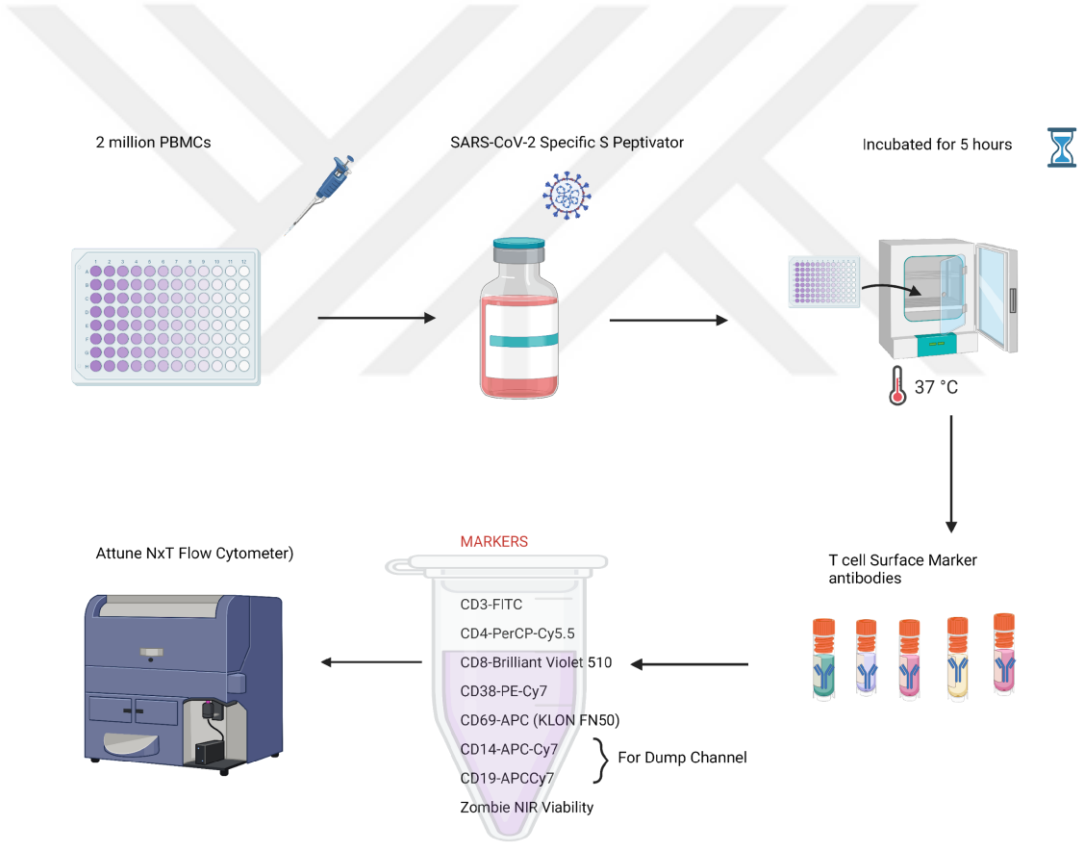


Figure 2.3: Flow Cytometry Steps for T cell Response (Created by Biorender, 2022).

2.7 Investigation of IFN-γ and IL-2 cytokine response by ELISpot Method

The IFN-γ and IL-2 responses of T cells were measured with Fluorospot Assay (Abcam Fluorospot, USA) according to the manufacturer's instructions. Plates were incubated for 30 seconds at room temperature with 35% ethanol and washed three times with DPBS 1X (Biowest, L0615-500). 100 µL of IFNγ capture antibody and 100 µL of IL-2 capture antibody were added to the 10 mL of PBS. 100 ul mixture was dispersed into each well, and the plate was covered and incubated overnight at +4°C. After washing with DPBS 1X (Biowest, L0615-500), RPMI-1640 medium (Sigma, R8758) containing 10% FBS cell culture media was added to the wells and incubated for 2 hours at room temperature (RT). Plates were washed with DPBS 1X and 10^5 PBMCs were added into each well with an appropriate concentration of SARS-CoV-2 S peptide activator (PepTivator®SARS-CoV-2 Prot S-research grade (6nmol/peptide, Miltenyi Biotec, Germany). The plate was covered with a standard 96-well plate plastic lid and cells were incubated at 37°C in a CO2 incubator for 20 hours and were washed 3 times using 1X DPBS containing 20% Tween-20 (P9416, Sigma). The FITC-labelled and biotinylated detection antibodies were added (for IFN-γ/IL-2 respectively) and incubated for 1 hour and 30 minutes at room temperature away from light. Following the wash step, FITC-green fluorescence conjugate/Streptavidin-phycoerythrin solution (for IFN-γ/IL-2 respectively) was added to each well and incubated for 1 hour at RT in the dark.

Plates were washed three times with PBS-0.05% Tween 20. The plate bottom was peeled and washed three times on both sides of the membrane under running distilled water. All residual buffer was removed by repeated tapping on absorbent paper.

After blot drying the plates, spots were read with GFP and RFP filters, under a dissection microscope (Leica M205 FA). Green, fluorescent spots indicate IFNγ production while IL-2 is revealed by red spots. Yellow spots indicate dual cytokine-producing cells. Plates were stored at +4°C away from light. Fluorescence is read immediately to prevent fade (Figure 2.4).

The PMA/Ionomycin was used as positive control while DMSO was used as a negative control. The tests were performed in duplicate (Table 2.2).

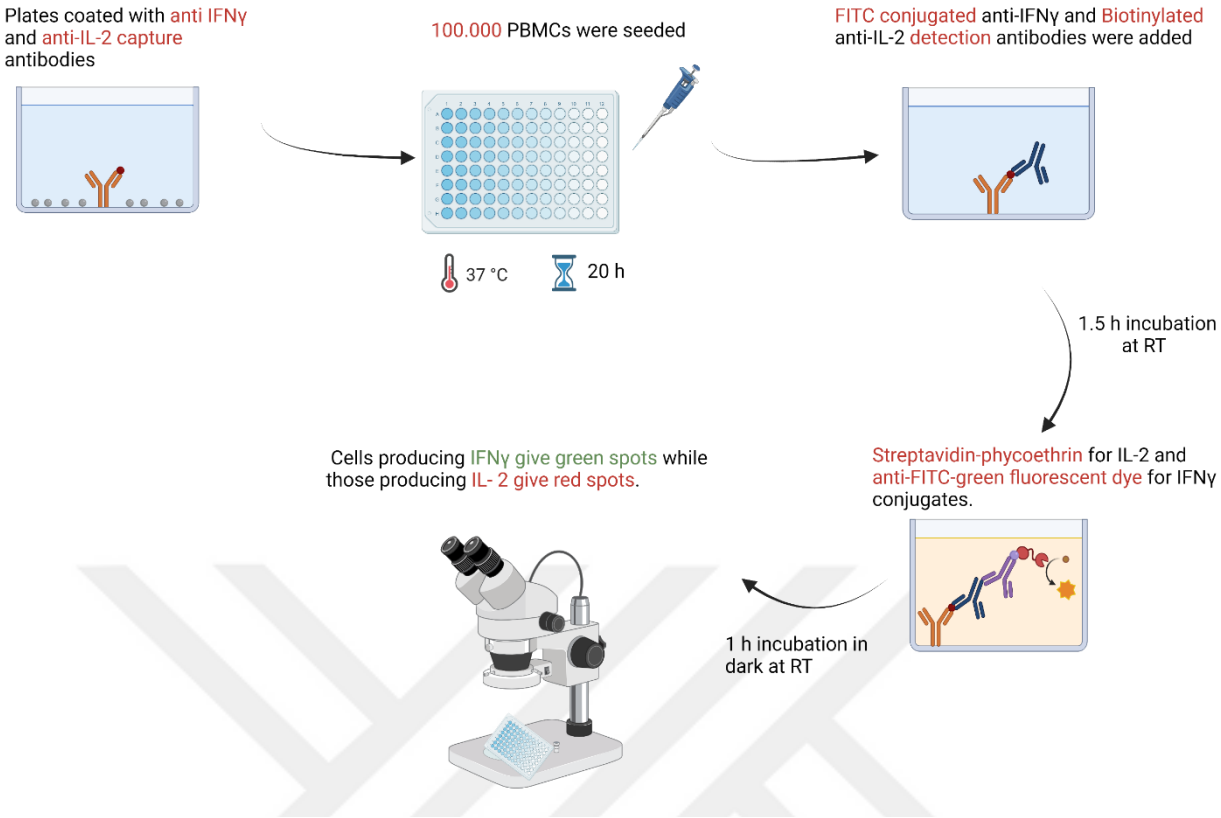


Figure 2.4: Elispot Assay Illustration (Created by Biorender, 2022).

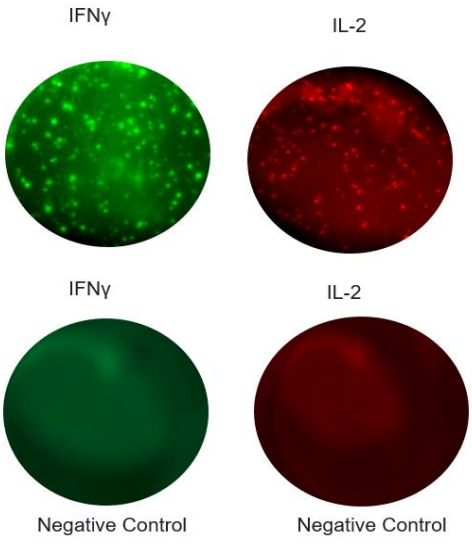


Figure 2.5: Spots under the dissection microscope by fluorescent light.

2.8 Statistical Analysis

After obtaining adequate longitudinal data, STATA was used to perform a statistical analysis of paired samples using a non-parametric test (Mann Whitney U) for the comparison of two groups (16v, USA). When the individuals were not accessible, an unpaired Mann Whitney U test was used. The acquired data were analyzed and visualized using GraphPad Prism 8.0.2 software. P =005 was the statistical significance cut-off point. (Bertoletti et al., 2021 , Tan et al., 2021)

CHAPTER 3:
RESULTS

3.1 T Cell Response

Phenotyping of S-specific $CD4^+$ and $CD8^+$ T cells was used to assess the effector T cell response after COVID-19 vaccination booster doses. Figure 3.1 depicts an example of gating.

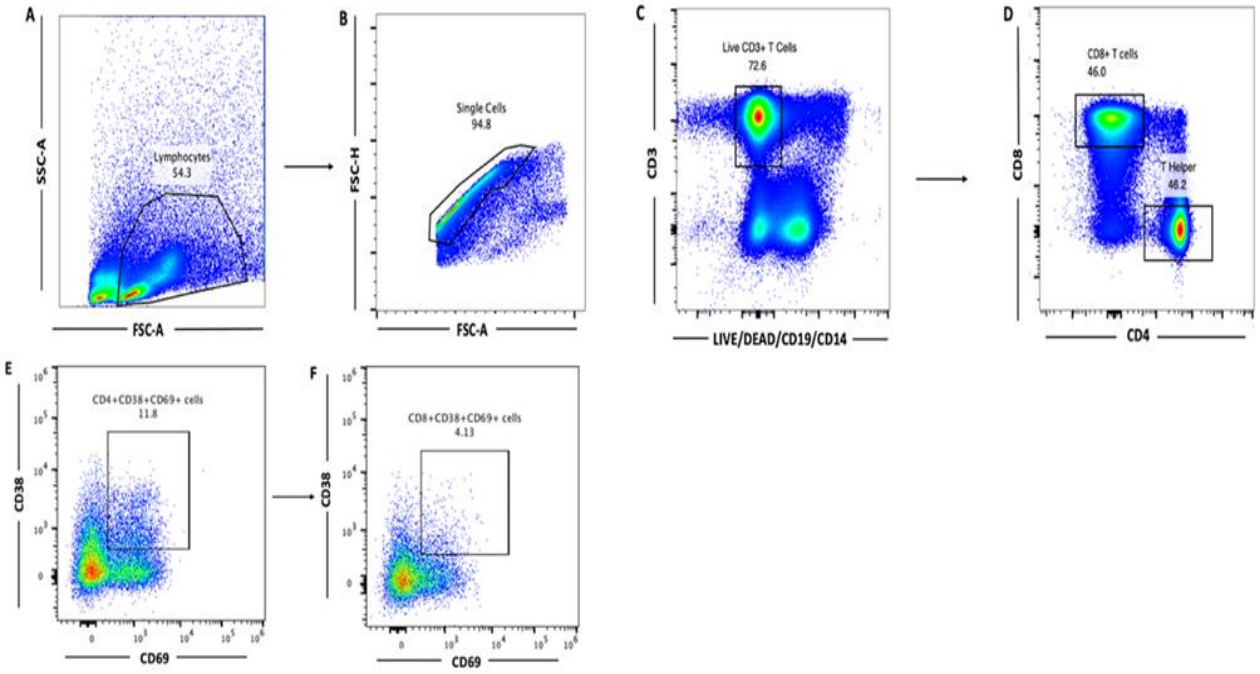


Figure 3.1: The gating strategy of T lymphocytes in flow cytometry.

(A) Lymphocytes were identified as SSC-A vs. FSC-A.
(B) Doublets were excluded with FSC-W vs. FSC-A gating.
(C) Live splenocytes were negative for the live/dead marker, CD19+, and CD14+ markers.

$CD4^+$ and $CD8^+$ T cell responses to the SARS-CoV-2 spike S peptide pool were investigated for reactivity and magnitude. There was no significant difference in the magnitude of $CD4^+$ and $CD8^+$ T cells after BNT162b2 and CV boosters compared to baseline levels in the assessment of $CD4^+$ and $CD8^+$ cell ratios after stimulation with a mixture of spiked peptides. The median of $CD4^+$ T cells in baseline is 55; after a CV booster dosage at 1^{st} month, it increased to 63 while at 3^{rd} month it decreased to 53. After BNT162b2 booster at 1^{st} month, the median of the $CD4^+$ ratio has been decreased to 53, while at 3^{rd} months it increased to 64 (Figure 3.2A).

Likewise, the ratio of $CD8^+$ T median was 26 after two doses of CV, however after CV booster dosage at 1^{st} month it decreased to 24 and increased to 26 at 3^{rd} months. When it comes to BNT162b2 booster, it increased to 27 at 1^{st} month and 28 at 3^{rd} months (Figure 3.2B).

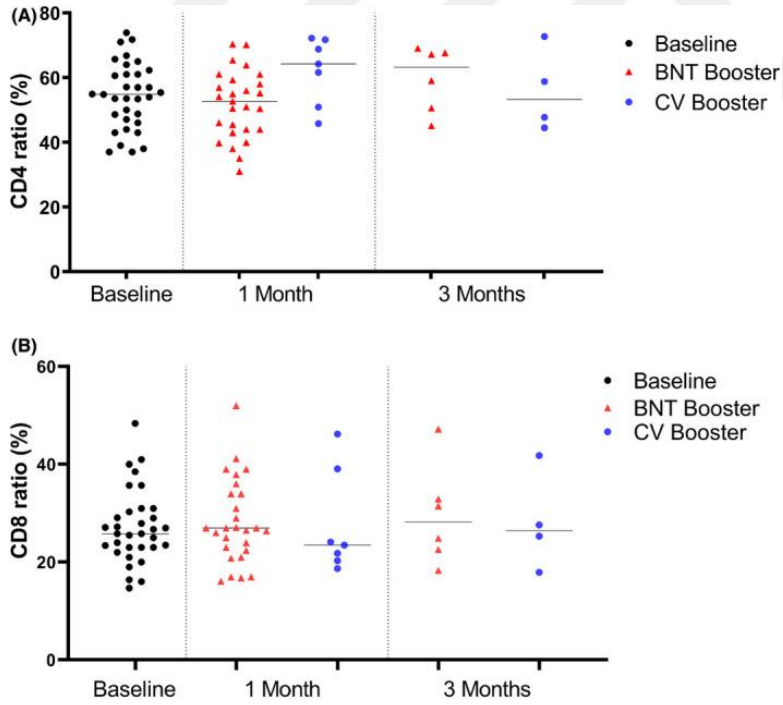
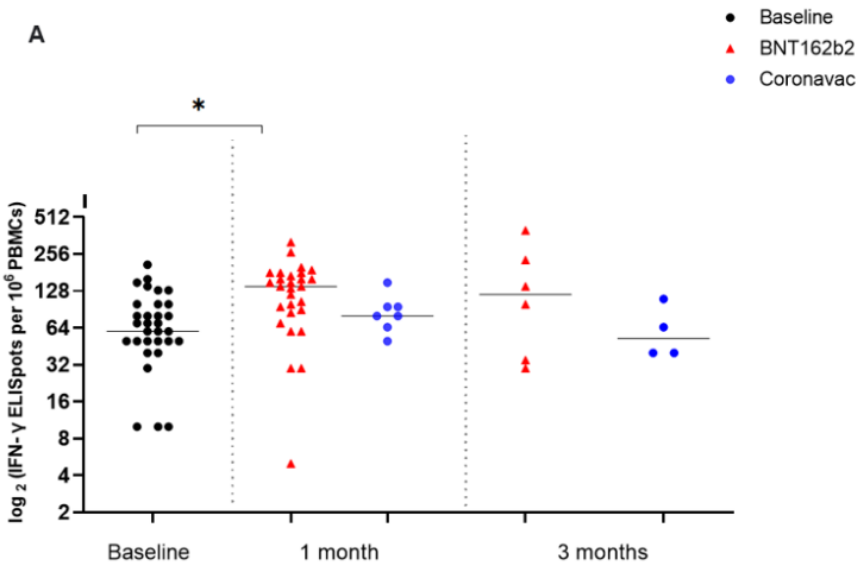


Figure 3.2: The proportion of specific T cell subpopulations after activation with SARS-CoV-2 S peptide pool.

A-CD4⁺ cell ratio at baseline, one month, and three months after boosters. **B-** CD8⁺ at baseline, one month, and three months after boosters. Dots represent T cell ratios for individuals in the population. Black lines are the median values of each population (Kuloglu & El et al., 2022).

T cells demonstrated significantly increased IFN-reactivity after a booster dosage in the ELISpot assay, with a median of 140 SFU per million PBMC (range 5 SFU-320 SFU) compared to baseline T cell response (60 SFU/10^6 PBMC; $p<0.001$). The IFN-reactivity of T cells (80 SFU/10^6 PBMC) did not alter significantly as a result of the CV booster dose (P=0.747). T cell IL-2 reactivity (60 SFU/106 PBMC) was considerably higher a month after BNT162b2 booster than CV booster (median SFU 30 /10^6 PBMC, $p<0.05$) and baseline levels (20 SFU/10^6 PBMC, $P<0.001$) (Figure 3.3).



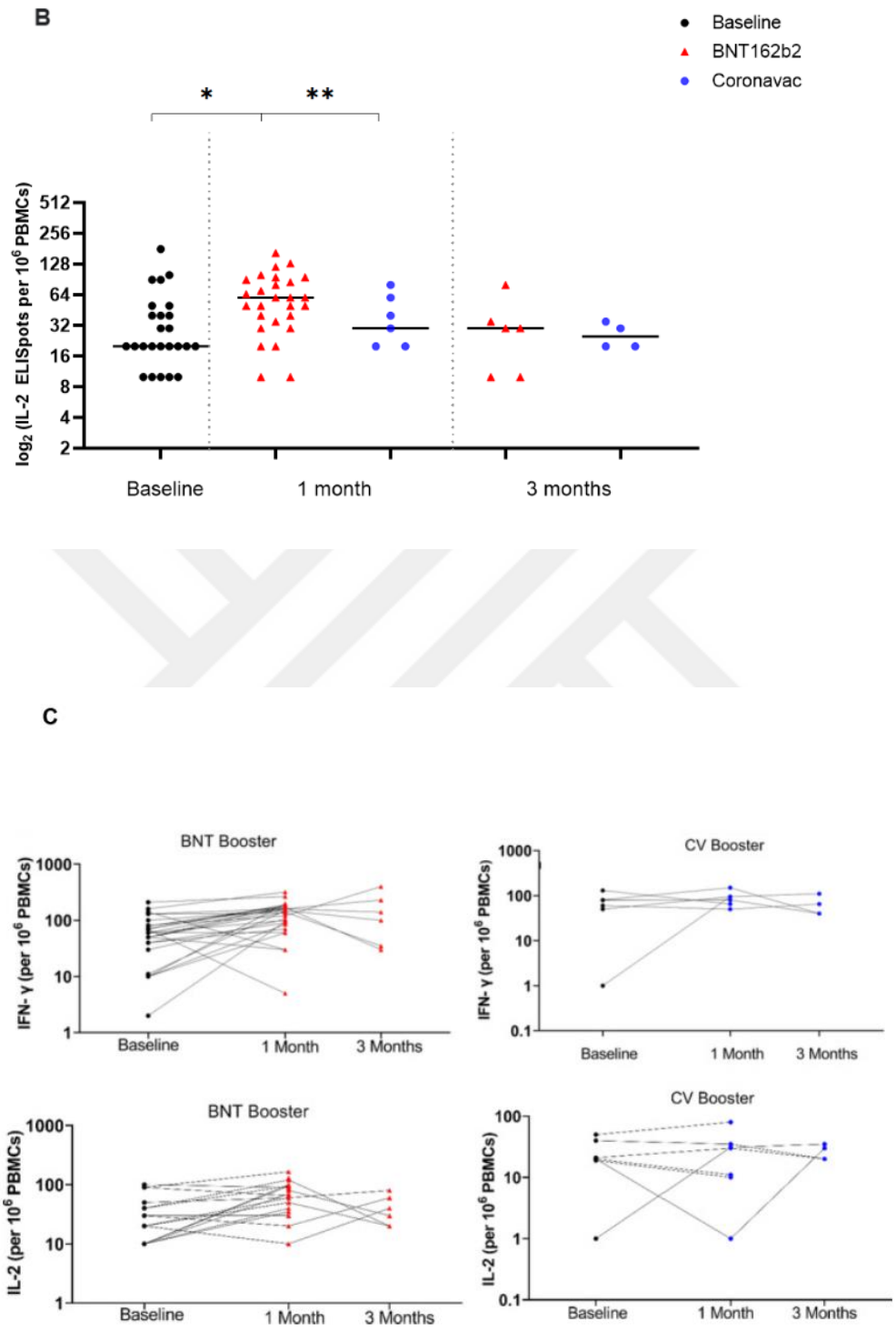
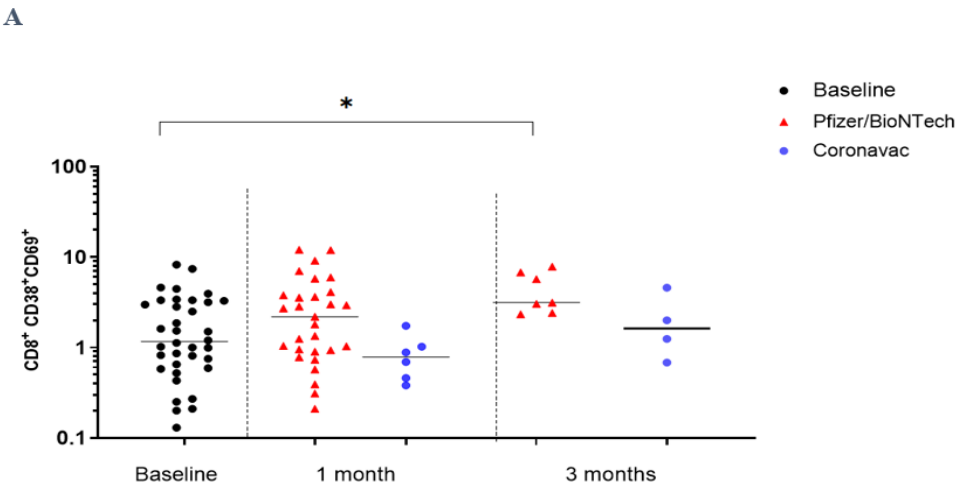


Figure 3.3: The ELISPOT results of T cells after incubation with the SARS-CoV-2 S peptide pool.

A- IFN-γ response at baseline, one month, and three months after boosters. **B-** IL-2 response at baseline, one month, and three months after boosters. Dots represent T cell ratios for individuals in the population. **C-** Changes in IFN- γ and IL-2 levels for each individual during a 3-month period. Dots represent IFN-γ and IL-2 levels for individuals in the population, (*P<0.001; **P<0.05) (Kuloglu & El et al., 2022).

Both $CD8^+CD38^+CD69^+$ T cells and $CD4^+CD38^+CD69^+$ T cells were activated one month after the BNT162b2 booster. In comparison to baseline levels, there was no significant difference, but in the third month, there was a considerable increase. After three months, the proportion of $CD8^+CD38^+CD69^+$ T cells grew from 1.160 percent to 3.130 percent (p=0.031), while the proportion of $CD4^+CD38^+CD69^+$ T cells increased from 4.37 percent to 10% (p=0.013). $CD4^+CD38^+CD69^+$ T cells increased from 4.37 percent to 10.20 percent (p=0.12) three months following the CV booster, while $CD8^+CD38^+CD69^+$ T cells increased from 1.160 percent to 3.130 percent (p=0.62) (Figure 3.4). (Kuloglu & El et al., 2022).



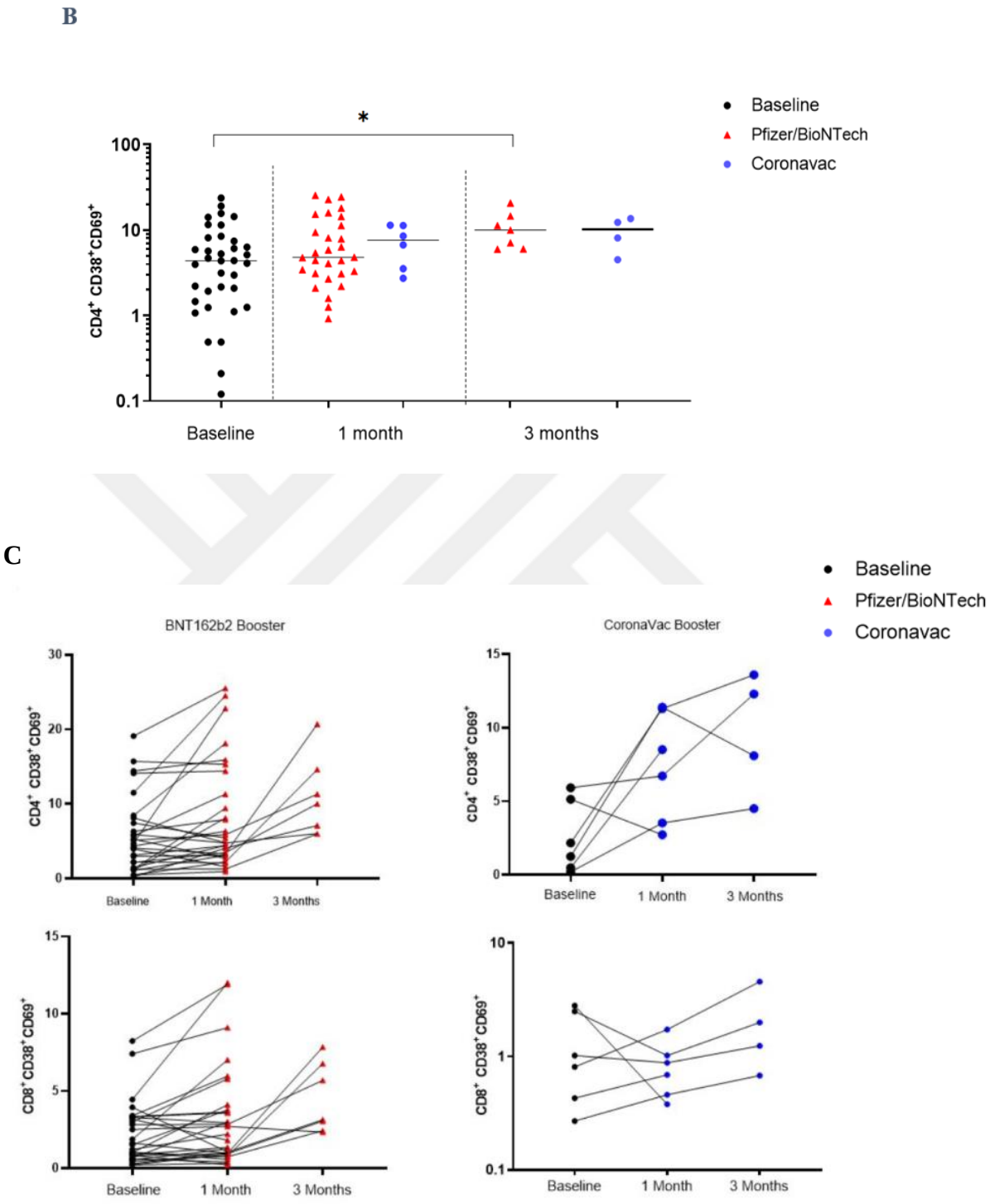


Figure 3.4: The ratio of CD8+CD38+CD69+ and CD4+CD38+CD69+ cells after BNT162b2 and CoronaVac boosters.

A- $CD8^+CD38^+CD69^+$ cell ratio at baseline, one month, and three months after boosters.
B- $CD4^+CD38^+CD69^+$ cell ratio at baseline, one month, and three months after boosters. Dots represent T cell ratios for individuals in the population.
C- Changes in the ratio of $CD8^+CD38^+CD69^+$ and $CD4^+CD38^+CD69^+$ cells for each individual during a 3-month period. Dots represent the ratio of $CD8^+CD38^+CD69^+$ and $CD4^+CD38^+CD69^+$ cells for individuals in the population (*P<0.05) (Kuloglu & El et al., 2022).

CHAPTER 4:
DISCUSSION

The purpose of this study is to fill in the gaps in the literature about the T cell response of BNT162b and CV boosters after two doses of CV regimen. T cell responses were investigated after one and three months evoked by BNT162b and CV boosters in individuals vaccinated with 2 doses of CV. Current vaccine studies have focused on stimulating neutralizing antibodies against SARS CoV-2, although $CD4^+$ and $CD8^+$ cells may also protect against severe disease and aid in COVID-19 clearance (Tan et al., 2021) (Geers, 2021).

Following the BNT162b booster, we noticed increased IFN-γ and IL-2 reactivity, as well as an increase in $CD8^+CD38^+CD69^+$ T cells. The IFN-γ reactivity of T cells did not change significantly after the CV booster. Previous studies also reported high T cell responses following the BNT162b booster in completely immunized healthy adults (Kanokudom et. al., 2021, Jantarabenjakul et. al., 2022 and Mellinghoff et. al .,2022). In accordance with our results, studies show that upon BNT162b vaccination in vitro stimulation with an overlapping peptide pool resulted in a strong activation of spike-specific $CD8^+$ T lymphocytes secreting IFN-γ, tumor necrosis factor TNF, and interleukin IL-2 (Li et. al., 2022). Moreover, they revealed that after second dose of BNT162b immunization, the quantity of the antigen specific $CD8^+$ T cells in the lung and spleen significantly increased. In our study, after the third month of the BNT162b booster, the ratio of effector $CD4^+CD38^+CD69^+$ cells were also high. Previous studies have revealed strong $CD8^+$ and $CD4^+$ T cells responses to BNT162b (Sahin et. al., 2021 and Kanokudom et. al., 2021) Also, a study done in Italy showed that Despite the fact that half of the participants did not produce a T-cell effector response after the booster dosage of BNT162b , all of them had SARS-CoV-2-specific T cells, indicating the maintenance of a long-lived memory T-cell compartment. Also they asserted that Shortening the period between doses may boost the vaccine's effectiveness on the T-cell response (Busà et. al., 2022).

Because effector T cells have a short lifespan, the increased ratio of $CD4^+$ to $CD8^+$ effector T cells in the third month after BNT162b immunization, was likely attributable to memory cell differentiation in the population (Figure 3.2).Likewise, a study in university of Californa, San Diego has revealed that SARS-CoV-2 specific T cell diminished with a half-life of 3 to 5 months (Dan et. al., 2021).

There are limited studies on T cell response after inactivated vaccines. We did not observe a significant increase in T cell counts and activity. A study from Hong Kong reported the activation of T cell responses after CV immunization whereas they detected BNT162b2 vaccination produces higher humoral reactions than CoronaVac vaccination (Mok et al., 2021). In addition, after stimulation, TNF-alpha and IL-2 production by T cells in vaccination responders was similar between the CV and BNT162b2vaccine types and convalescent COVID-19 samples (Mok et al., 2021). This study did not explain the cause of T cell responses by CV. The difference between our results could be due to the genetic factors between ethnic groups.

Inactivated vaccines can also be used to induce T cell clones against other SARS-CoV-2 antigens, such as the N protein (Nguyen et al., 2021). T-cell response measures may have been influenced by the sort of peptide pool applied for activation. (Prendecki et al., 2021). Instead of using viral peptides, we have utilized S-pool in this study which may have resulted in an underestimating of the true magnitude of T-cell responses.

Our study's strength is that it is a longitudinal study with the added benefit of being conducted in Turkey, where the predominant vaccine schedule consisted of two CV doses. The study's main limitations are a small sample number for detecting statistical significance, particularly in the CoronaVac booster group. The magnitude of post-BNT162b booster T cell responses in our sample was larger than the CV booster. Our findings, however, are consistent with those of previous research in Turkey that used ELISA to assess anti-S antibody titers (Yalcin et al., 2021). Because participants received their fourth dose after two months, several of the individuals were dropped from the study. Furthermore, we were unable to cover the newly discovered Omicron variation, which will be addressed in future investigations. This project has significance for countries that have implemented a two-dose CV regimen. After the BNT162b

booster, the ratio of effector $CD8^+$ and $CD4^+$ T cells rose, as did IFN-γ activation. Given the waning immunity, a second BNT162b booster dose for those who have already had BNT162b or CV boosters as their third dose after two doses of CV is recommended.

Although promising, these findings are based on a small sample and a relatively short follow-up period after the booster dose. Long-term research is needed to determine the longevity and adaptability of the immunological memory established by vaccinations. The combined research of serum antibodies, memory B cells, effector T-cell response, and memory T cells should aid us in better understanding their time-related kinetics and duration, with the goal of improving current and future anti-SARS-CoV-2 vaccination techniques and decisions.

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APPENDIX A:

Table 0.1: ELISpot Assay Table

#	Vaccine Time	Type	IFN-gamma	IL-2
1	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable
1	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	17	1
1	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	20	13
1	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	21	15
1	2 doses of CoronaVac + BNT162b2+ 1 month	CMV	11	4
1	2 doses of CoronaVac + BNT162b2+ 1 month	Unstimulated	9	2
2	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable
2	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	6	3
2	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	20	10
2	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	21	7
2	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	3	1
2	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	6	1?

3	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable
3	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	8	0
3	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	11	2
3	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	25	2
3	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	6	12
3	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	0	0
4	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable
4	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	5	2
4	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	12	6
4	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	15	6
4	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	9	10
4	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	0	1
5	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable
5	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	15	3
5	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	18	4
5	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	19	7
5	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	24	7
5	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	2	0

6	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable
6	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	10	3
6	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	18	6
6	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	18	5
6	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	12	5
6	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	2	1
7	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable
7	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	?	4
7	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	36	1
7	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	40	4
7	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	51	2
7	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	30 (9 bright)	9 (1 bright)
8	2 doses of CoronaVac + 4 months	PMA	Uncountable	24
8	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	25	4
8	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	36	1
8	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	35	5
8	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	33	1
8	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	19 (6 bright)	2
9	2 doses of CoronaVac + 4 months	PMA	25	7

9	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	7	0
9	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	9	1
9	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	12	0
9	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	5	10
9	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	1	0
10	2 doses of CoronaVac + 4 months	PMA	100+	26
10	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	16	1
10	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	33	4
10	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	30	8
10	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	13	8
10	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	0	0
11	2 doses of CoronaVac + 4 months	PMA	55+	8
11	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	7	1
11	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	13	4
11	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	18	3
11	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	7	1
11	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	2	0
12	2 doses of CoronaVac + 4 months	PMA	100+	6

12	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	5	0
12	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	27	0
12	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	8	3
12	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	6	0
12	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	0	0
13	2 doses of CoronaVac + 4 months	PMA	50+	5
13	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	7	3
13	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	8	2
13	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	6	8
13	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	14	3
13	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	3	0
14	2 doses of CoronaVac + 4 months	PMA	50+	3
14	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	12	9
14	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	14	6
14	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	19	2
14	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	14	5
14	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	2	?
15	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable

15	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	8	7
15	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	11	18
15	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	15	13
15	2 doses of CoronaVac + BNT162b2+ 1 month	CMV	6	15
15	2 doses of CoronaVac + BNT162b2+ 1 month	Unstimulated	4	7
16	2 doses of CoronaVac + 4 months	PMA	Uncountable	15
16	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	8	0
16	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	23	6
16	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	15	18
16	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	14	3
16	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	3	2
17	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable
17	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	25	13
17	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	25	9
17	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	26	12
17	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	18	6
17	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	12	4
18	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable

18	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	30	6
18	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	38	11
18	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	33	9
18	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	11	9
18	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	9	7
19	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable
19	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	20	9
19	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	29	5
19	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	35	10
19	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	28	13
19	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	13	4
20	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable
20	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	22	12
20	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	13	4
20	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	18	16
20	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	22	17
20	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	15	8
21	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable

21	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	19	8
21	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	30	14
21	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	39	12
21	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	17	4
22	2 doses of CoronaVac + 4 months	PMA	Uncountable	18
22	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	6	3
22	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	10	3
	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	12	2
22	2 doses of CoronaVac + BNT162b2+ 1 month	CMV	8	3
22	2 doses of CoronaVac + BNT162b2+ 1 month	Unstimulated	5	2
23	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable
23	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	8	1
23	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	25	4
23	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	13	8
23	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	10	2
23	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	1	0
24	2 doses of CoronaVac + 4 months	PMA	Uncountable	15
24	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	18	10

24	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	9	5
24	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	5	9
24	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	11	2
24	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	4	0
25	2 doses of CoronaVac + 4 months	PMA	Uncountable	21
25	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	3	2
25	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	20	13
25	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	24	5
25	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	?	?
25	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	2	0
26	2 doses of CoronaVac + 4 months	PMA	Uncountable	Uncountable
26	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	6	4
26	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	12	17
26	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	13	16
26	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	4	2
26	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	9	0
27	2 doses of CoronaVac + 5 months	PMA	Uncountable	Uncountable

27	2 doses of CoronaVac + 5 months	SARS-CoV-2 peptivator	8	18
27	2 doses of CoronaVac + BNT162b2 + 15 days	SARS-CoV-2 peptivator	6	12
27	2 doses of CoronaVac + BNT162b2 + 15 days	SARS-CoV-2 peptivator	10	7
27	2 doses of CoronaVac + BNT162b2 + 15 days	CMV	18	0
27	2 doses of CoronaVac + BNT162b2 + 15 days	Unstimulated	2	0
28	Healthy Control	PMA	Uncountable	8
28	Healthy Control	SARS-CoV-2 peptivator	8	6
28	Healthy Control	SARS-CoV-2 peptivator	15	10
28	Healthy Control	CMV	18	12
28	Healthy Control	Unstimulated	0	0
29	2 doses of CoronaVac + 5 months	PMA	Uncountable	Uncountable
29	2 doses of CoronaVac + 5 months	SARS-CoV-2 peptivator	5	3
29	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	9	13
29	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	16	8
29	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	2	1
30	2 doses of CoronaVac + 5 months	PMA	Uncountable	Uncountable
30	2 doses of CoronaVac + 5 months	SARS-CoV-2 peptivator	3	3
30	2 doses of CoronaVac + BNT162b2 + 15 days	SARS-CoV-2 peptivator	8	2
30	2 doses of CoronaVac + BNT162b2 + 15 days	SARS-CoV-2 peptivator	13	10

30	2 doses of CoronaVac + BNT162b2 + 15 days	Unstimulated	2	1
31	Just before 3rd dose of CoronaVac	PMA	Uncountable	Uncountable
31	Just before 3rd dose of CoronaVac	SARS-CoV-2 peptivator	6	3
31	3 doses of CoronaVac + 1 month	SARS-CoV-2 peptivator	7	3
31	3 doses of CoronaVac + 1 month	SARS-CoV-2 peptivator	11	5
31	3 doses of CoronaVac + 1 month	Unstimulated	1	1
32	Just before 3rd dose of CoronaVac	PMA	Uncountable	Uncountable
32	Just before 3rd dose of CoronaVac	SARS-CoV-2 peptivator	9	4
32	3 doses of CoronaVac + 1 month	SARS-CoV-2 peptivator	9	3
32	3 doses of CoronaVac + 1 month	SARS-CoV-2 peptivator	12	3
32	3 doses of CoronaVac + 1 month	Unstimulated	1	2
33	Just before 3rd dose of CoronaVac	PMA	Uncountable	Uncountable
33	Just before 3rd dose of CoronaVac	SARS-CoV-2 peptivator	12	5
33	3 doses of CoronaVac + 1 month	SARS-CoV-2 peptivator	8	9
33	3 doses of CoronaVac + 1 month	SARS-CoV-2 peptivator	9	7
33	3 doses of CoronaVac + 1 month	Unstimulated	2	0
34	Just before 3rd dose of CoronaVac	PMA	Uncountable	Uncountable
34	Just before 3rd dose of CoronaVac	SARS-CoV-2 peptivator	13	2

34	3 doses of CoronaVac + 1 month	SARS-CoV-2 peptivator	12	1
34	3 doses of CoronaVac + 1 month	SARS-CoV-2 peptivator	13	4
34	3 doses of CoronaVac + 1 month	Unstimulated	3	2
35	Just before 3rd dose of CoronaVac	PMA	Uncountable	Uncountable
35	Just before 3rd dose of CoronaVac	SARS-CoV-2 peptivator	15	4
35	3 doses of CoronaVac + 1 month	SARS-CoV-2 peptivator	16	3
35	3 doses of CoronaVac + 1 month	SARS-CoV-2 peptivator	14	4
35	3 doses of CoronaVac + 1 month	Unstimulated	0	0
36	Just before 3rd dose of CoronaVac	PMA	50+	18
36	Just before 3rd dose of CoronaVac	SARS-CoV-2 peptivator	5	2
36	3 doses of CoronaVac + 1 month	SARS-CoV-2 peptivator	7	2
36	3 doses of CoronaVac + 1 month	SARS-CoV-2 peptivator	3	2
36	3 doses of CoronaVac + 1 month	Unstimulated	0	0
37	Healthy Control	PMA	Uncountable	Uncountable
37	Healthy Control	SARS-CoV-2 peptivator		
37	Healthy Control	SARS-CoV-2 peptivator		
37	Healthy Control	SARS-CoV-2 peptivator		
37	Healthy Control	Unstimulated		
38	2 doses of CoronaVac + 4 months	PMA	50+	Uncountable

38	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	6	5
38	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	10	7
38	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	8	5
38	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	3	3
39	Just before 3rd dose of CoronaVac	PMA	Uncountable	Uncountable
39	Just before 3rd dose of CoronaVac	SARS-CoV-2 peptivator	6	1
39	3 doses of CoronaVac + 1 month	SARS-CoV-2 peptivator	8	2
39	3 doses of CoronaVac + 1 month	SARS-CoV-2 peptivator	3	0
39	3 doses of CoronaVac + 1 month	Unstimulated	3	1
40	2 doses of CoronaVac + BNT162b2 + 3 months	PMA	Uncountable	Uncountable
40	2 doses of CoronaVac + BNT162b2 + 3 months	SARS-CoV-2 peptivator	10	0
40	2 doses of CoronaVac + BNT162b2 + 3 months	SARS-CoV-2 peptivator	13	2
40	2 doses of CoronaVac + BNT162b2 + 3 months	Unstimulated	0	0
41	2 doses of CoronaVac + BNT162b2 + 3 months	PMA	Uncountable	Uncountable
41	2 doses of CoronaVac + BNT162b2 + 3 months	SARS-CoV-2 peptivator	6	5
41	2 doses of CoronaVac + BNT162b2 + 3 months	SARS-CoV-2 peptivator	13	5

41	2 doses of CoronaVac + BNT162b2 + 3 months	Unstimulated	7	5
42	2 doses of CoronaVac + BNT162b2 + 3 months	PMA	Uncountable	Uncountable
42	2 doses of CoronaVac + BNT162b2 + 3 months	SARS-CoV-2 peptivator	6	4
42	2 doses of CoronaVac + BNT162b2 + 3 months	SARS-CoV-2 peptivator	5	0
42	2 doses of CoronaVac + BNT162b2 + 3 months	Unstimulated	1	1
43	2 doses of CoronaVac + BNT162b2 + 3 months	PMA	Uncountable	Uncountable
43	2 doses of CoronaVac + BNT162b2 + 3 months	SARS-CoV-2 peptivator	30	6
43	2 doses of CoronaVac + BNT162b2 + 3 months	SARS-CoV-2 peptivator	25	5
43	2 doses of CoronaVac + BNT162b2 + 3 months	Unstimulated	15	3
44	2 doses of CoronaVac + BNT162b2 + 3 months	PMA	Uncountable	Uncountable
44	2 doses of CoronaVac + BNT162b2 + 3 months	SARS-CoV-2 peptivator	8	6
44	2 doses of CoronaVac + BNT162b2 + 3 months	SARS-CoV-2 peptivator	Couldn’t count(spots were blurry)	0
44	2 doses of CoronaVac + BNT162b2 + 3 months	Unstimulated	5	2
45	2 doses of CoronaVac + BNT162b2 + 3 months	PMA	Uncountable	Uncountable

45	2 doses of CoronaVac + BNT162b2 + 3 months	SARS-CoV-2 peptivator	8	1
45	2 doses of CoronaVac + BNT162b2 + 3 months	SARS-CoV-2 peptivator	9	2
45	2 doses of CoronaVac + BNT162b2 + 3 months	Unstimulated	3	1
46	2 doses of CoronaVac + 5 months	PMA	Uncountable	21
46	2 doses of CoronaVac + 5 months	SARS-CoV-2 peptivator	3	2
46	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	20	13
46	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	24	5
46	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	check	check
46	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	2	0
47	2 doses of CoronaVac + 5 months	PMA	Uncountable	Uncountable
47	2 doses of CoronaVac + 5 months	SARS-CoV-2 peptivator	6	4
47	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	12	17
47	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	13	16
47	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	4	2
47	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	9	0
48	2 doses of CoronaVac + 5 months	PMA	Uncountable	Uncountable
48	2 doses of CoronaVac + 5 months	SARS-CoV-2 peptivator	8	18
48	2 doses of CoronaVac + BNT162b2 + 15 days	SARS-CoV-2 peptivator	6	12

48	2 doses of CoronaVac + BNT162b2 + 15 days	SARS-CoV-2 peptivator	10	7
48	2 doses of CoronaVac + BNT162b2 + 15 days	CMV	18	0
48	2 doses of CoronaVac + BNT162b2 + 15 days	Unstimulated	2	0
49	2 doses of CoronaVac + 4 months	PMA	U ncountable	Uncountable
49	2 doses of CoronaVac + 4 months	SARS-CoV-2 peptivator	22	12
49	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	13	4
49	2 doses of CoronaVac + BNT162b2 + 1 month	SARS-CoV-2 peptivator	18	16
49	2 doses of CoronaVac + BNT162b2 + 1 month	CMV	22	17
49	2 doses of CoronaVac + BNT162b2 + 1 month	Unstimulated	15	8
50	2 doses of CoronaVac + 5 months	PMA	Uncountable	Uncountable
50	2 doses of CoronaVac + 5 months	SARS-CoV-2 peptivator	3	3
50	2 doses of CoronaVac + BNT162b2 + 15 days	SARS-CoV-2 peptivator	8	2
50	2 doses of CoronaVac + BNT162b2 + 15 days	SARS-CoV-2 peptivator	13	10
50	2 doses of CoronaVac + BNT162b2 + 15 days	Unstimulated	2	1
51	2 doses of CoronaVac + BNT162b2 + 3 months	PMA	3	2
51	2 doses of CoronaVac + BNT162b2 + 3 months	SARS-CoV-2 peptivator	5	9
51	2 doses of CoronaVac + BNT162b2 + 3 months	SARS-CoV-2 peptivator	2	3
51	2 doses of CoronaVac + BNT162b2 + 3 months	Unstimulated	0	0

52	3 doses of CoronaVac + 3 months	PMA	40+	3
52	3 doses of CoronaVac + 3 months	SARS-CoV-2 peptivator	7	2
52	3 doses of CoronaVac + 3 months	SARS-CoV-2 peptivator	6	4
52	3 doses of CoronaVac + 3 months	Unstimulated	0	0
53	3 doses of CoronaVac + 3 months	PMA	40+	0
53	3 doses of CoronaVac + 3 months	SARS-CoV-2 peptivator	17	4
53	3 doses of CoronaVac + 3 months	SARS-CoV-2 peptivator	7	3
53	3 doses of CoronaVac + 3 months	Unstimulated	2	0
54	3 doses of CoronaVac + 3 months	PMA	20+	0
54	3 doses of CoronaVac + 3 months	SARS-CoV-2 peptivator	3	2
54	3 doses of CoronaVac + 3 months	SARS-CoV-2 peptivator	5	0
54	3 doses of CoronaVac + 3 months	Unstimulated	0	0
55	3 doses of CoronaVac + 3 months	PMA	20+	0
55	3 doses of CoronaVac + 3 months	SARS-CoV-2 peptivator	3	0
55	3 doses of CoronaVac + 3 months	SARS-CoV-2 peptivator	3	2
55	3 doses of CoronaVac + 3 months	Unstimulated	2	0

Table 0.2 Flow Assay CD8+CD38+ and CD4+CD38+ Table for BNT162b2 Booster.

#	2 doses of CoronaVac + 4 months		2 doses of CoronaVac + BNT162b2 + 1 month	
	CD4+CD38+	CD8+CD38+	CD4+CD38+	CD8+CD38
1	7.76%	1.52%	8.01%	1.42%
2	14%	2%	16%	2%
3	0.05%	0.03%	14%	1.65%
4	19%	3%	23%	5.04%
5	18%	3.50%	20%	5%
6	10.20%	2.16%	16%	3.70%
7	18%	3.66%	18%	4%
8	17%	5.20%	22%	5.40%
9	17.70%	4.63%	19.60%	4.64%
10	28.80%	6.21%	23.80%	6.07%
11	37.70%	7.77%	27.20%	5.28%
12	19.40%	2.54%	21.40%	3.66%
13	7.85%	1.03%	7.07%	1.31%
14	15.10%	3.93%	21.60%	4.84%
15	7%	4%	11%	10.50%
16	7%	2%	9%	3%
17	8%	3%	10%	3%
18	3.57%	11%	4.23%	13%
19	3.70%	17.70%	2.15%	11.10%
20	13%	4%	12.50%	5%
21	14.10%	22.40%	5.74%	16.70%
22	7%	2.40%	7%	6%

#	2 doses of CoronaVac + 4 months		2 doses of CoronaVac + BNT162b2 + 1 month	
	CD4+CD38+	CD8+CD38+	CD4+CD38+	CD8+CD38
1	7.76%	1.52%	8.01%	1.42%
2	14%	2%	16%	2%
3	0.05%	0.03%	14%	1.65%
4	19%	3%	23%	5.04%
23	2.12%	13.50%	2.97%	17.20%
24	8%	4%	4%	3%
25	1.71%	2.60%	3.34%	5.36%
26	1.40%	7.59%	3%	10%
27	10%	3%	9%	2%
28	23.70%	4.42%	43.40%	8.49%
29	9%	4%	-	-

Table 0.3: Flow Assay CD8+CD69+ and CD4+CD69+ Table for BNT162b2 Booster.

#	2 doses of CoronaVac + 4 months		2 doses of CoronaVac + BNT162b2 + 1 month	
	CD4+CD69+	CD8+CD69+	CD4+CD69+	CD8+CD69
1	37%	17%	37%	15%
2	3%	3%	45%	26%
3	30%	12%	60%	23%
4	65%	49%	40%	25%
5	60%	34%	54%	25%
6	13%	8.50%	25%	7%
7	45%	21%	38%	12%
8	51%	34%	57%	30%
9	15%	13.50%	47.80%	34.20%
10	49.50%	47.20%	70.40%	70.3
11	17.90%	19.40%	40.50%	28.80%
12	16.80%	22.20%	45.60%	39.60%
13	44.60%	35%	52.00%	45.60%
14	49.30%	40.40%	38.80%	33.30%

15	22%	11%	36%	24%
16	26%	7%	26%	10%
17	5%	2.20%	35%	17%
18	21.40%	12.20%	42.70%	24.30%
19	26.20%	12.70%	27.30%	18.10%
20	36%	14%	44%	27%
21	15.80%	18.10%	27.70%	14.50%
22	29%	11%	31%	12%
23	37.10%	37.60%	24.10%	11.50%
24	9%	5%	8%	4%
25	40.70%	24.10%	35.5	14.7
26	21.70%	14.50%	41%	32%
27	17.10%	17%	23%	21%
28	39%	34%	51%	36%
29	32.40%	14.30%	-	-

Table 0.4: Flow Assay CD8+ TNF-alpha and CD4+ TNF- alpha Table for BNT162b2 Booster.

#	2 doses of CoronaVac + 4 months		2 doses of CoronaVac + BNT162b2 + 1 month	
	CD4+ TNF -alpha	CD8+ TNF-alpha	CD4+ TNF- alpha	CD8+ TNF-alpha
1	0.03%	0.02%	0.02%	0.02%
2	0.04%	0.03%	0.02%	0.03%
3	0.02%	2%	0.04%	0.25%
4	0.03%	0.06%	0.03%	0.03%
5	0.03%	0.04%	0.02%	0.03%
6	0.02%	0.02%	0.03%	0.02%
7	0.04%	0.03%	0.02%	0.03%
8	0.32%	0.13%	0.03%	0.03%
9	0.46%	0.54%	0.16%	0.31%
10	0.32%	0.54%	0.49%	0.73%
11	0.21%	0.38%	0.24%	0.36%
12	0.22%	0.44%	0.23%	0.37%
13	0.34%	0.54%	0.21%	0.37%
14	0.19%	0.36%	0.18%	0.39%

15	0.30%	0.10%	0.57%	0.15%
16	0.41%	0.21%	0.42%	0.34%
17	0.44%	0.10%	0.20%	0.20%
18	0.16%	0.34%	0.01%	0.14%
19	0.15%	0.40%	0.14%	0.39%
20	0.40%	0.45%	0.14%	0.18%
21	0.16%	0.79%	0.17%	0.53%
22	1%	1%	0.23%	0.35%
23	0.26%	0.60%	0.14%	0.29%
24	0.10%	0.15%	0%	1%
25	0.24%	0.61%	0.20%	0.23%
26	0.07%	0.01%	0.01%	0.27%
27	1.09%	1.40%	1.05%	1.32%
28	0.66%	1%	1.09%	1.40%
29	0.40%	0.10%	-	-

Table 0.5: Flow Assay CD8+ IFN-gamma and CD4+ IFN-gamma Table for BNT162b2 Booster.

#	2 doses of CoronaVac + 4 months		2 doses of CoronaVac + BNT162b2 + 1 month	
	CD4+ IFN gamma	CD8+IFN gamma	CD4+ IFN gamma	CD8+IFN gamma
1	0.04%	0.00%	0.02%	0.02%
2	0.18%	0.05%	0.07%	0.02%
3	0.05%	0.03%	0.12%	0.01%
4	0.51%	0.05%	0.56%	0.03%
5	0.28%	0.04%	0.36%	0.02%
6	0.15%	0.01%	0.13%	0.01%
7	0.21%	0.03%	0.14%	0.00%
8	0.73%	0.85%	0.32%	0.01%
9	0.69%	0.26%	0.50%	0.18%
10	0.33%	0.37%	1.43%	2.26%
11	0.65%	0.20%	0.76%	0.31%
12	0.47%	2.31%	0.41%	0.19%
13	0.37%	0.42%	0.57%	0.55%
14	0.56%	0.23%	0.28%	0.32%
15	4%	0.12%	4.10%	0.70%

16	4%	0.12%	3%	11%
17	53%	0.05%	4%	0.24%
18	0.13%	1.20%	0.12%	0.44%
19	0.05%	0.53%	0.05%	0.34%
20	4%	0.10%	4%	0.02%
21	0.24%	3.27%	0.22%	1.51%
22	4%	0.48%	3%	0.10%
23	0.07%	0.30%	0.08%	0.44%
24	6%	0.10%	10%	0%
25	0.29%	0.96%	0.23%	1.05%
26	0.07%	0.83%	0.06%	0.33%
27	0.11%	0.10%	0.10%	0.08%
28	0.21%	0.16%	0.26%	0%
29	2.40%	0.10%	-	-

Table 0.6: Flow Assay CD8+ IL-2 and CD4+ IL-2 Table BNT162b2 Booster.

#	2 doses of CoronaVac + 4 months		2 doses of CoronaVac + BNT162b2 + 1 month	
	CD4+ IL-2	CD8+IL-2	CD4+ IL-2	CD8+IL-2
1	0.01%	0.07%	0.02%	1.17%
2	0.02%	1.20%	0.01%	1.90%
3	31%	12.50%	0.02%	1.75%
4	0.02%	2.19%	0.01%	2.61%
5	0.03%	1.78%	0.02%	2.80%
6	0.02%	1.20%	0.03%	1.80%
7	0.02%	2.25%	0.02%	2.12%
8	0.63%	1.17%	0.01%	1.17%
9	0.11%	0.36%	0.15%	0.39%
10	0.09%	0.41%	0.14%	0.35%
11	0.09%	0.33%	0.09%	0.26%
12	0.07%	0.26%	0.09%	0.23%
13	0.17%	0.44%	0.13%	0.26%
14	0.12%	0.19%	0.07%	0.39%
15	1%	2%	0.02%	3%
16	0.03%	2.31%	0.03%	4%

17	0.01%	1%	0.01%	3%
18	0.21%	0%	0.19%	0.02%
19	0.17%	12.70%	0.20%	0.03%
20	0.03%	3%	0.02%	4.10%
21	0.33%	0.03%	0.34%	0.03%
22	0.05%	3.50%	1%	5%
23	0.17%	0%	0.17%	0.03%
24	0.03%	2%	0%	0.50%
25	0.21%	0%	0.23%	1.05%
26	0.13%	3.57%	0.17%	0.03%
27	0.33%	0.14%	0.29%	0.15%
28	0.41%	0%	0%	0.94%
29	0.01%	-	-	-

Table 0.7: Flow Assay CD8+CD38+ and CD4+CD38+ Table for CoronoVac Booster.

	2 doses of CoronaVac + 4 months		3 doses of CoronaVac + 1 month	
	CD4+CD38+	CD8+CD38+	CD4+CD38+	CD8+CD38
1	19.50%	1.73%	15.30%	1.68%
2	11.10%	1.69%	12%	2.14%
3	1.65%	2%	17%	1.86%
4	4.50%	0.94%	6.18%	0.82%
5	30.50%	7.14%	17.50%	3.13%
6	4.48%	0.68%	4.89%	0.88%

Table 0.8: Flow Assay CD8+CD38+ and CD4+CD38+ Table for CoronoVac Booster.

	2 doses of CoronaVac + 4 months		3 doses of CoronaVac + 1 month	
	CD4+CD69+	CD8+CD69+	CD4+CD69+	CD8+CD69+
1	45.60%	39%	46.50%	36.80%
2	53%	32%	48.80%	30%
3	53%	53%	60%	50%

4	46%	35%	53%	32%
5	66%	56%	53%	33%
6	58.70%	49%	65%	49%

Table 0.9: Flow Assay CD8+TNF-alpha and CD4+TNF-alpha Table for CoronoVac Booster.

	2 doses of CoronaVac + 4 months		3 doses of Sinovac	
	CD4+ TNF alpha	CD8+ TNF-alpha	CD4+ TNF alpha	CD8+ TNF-alpha
1	0.685	0.73%	0.59%	1.06
2	0.51%	0.84%	0.63%	0.81%
3	0.92%	1.06%	0.96%	1.20%
4	0.51%	0.81%	0.63%	0.81%
5	0.89%	1.74%	0.84%	1.10%
6	0.75%	0.61%	0.44%	1.25%

Table 0.10: Flow Assay CD8+ IFN-gamma and CD4+ IFN-gamma Table for CoronoVac Booster.

	2 doses of CoronaVac + 4 months		3 doses of CoronaVac + 1 month	
	CD4+ IFN-gamma	CD8+IFN- gamma	CD4+ IFN-gamma	CD8+IFN-gamma
1	0.16%	0.01%	0.15%	0.09%
2	0.16%	0.17%	0.12%	0.07%
3	0.50%	0.15%	0.30%	0.27%
4	0.16%	0.17%	0.12%	0.07%
5	0.19%	0.165	0.75%	0.07%
6	0.32%	0.18%	0.24%	0.28%

Table 0.11: Flow Assay CD8+ IL-2 and CD4+ IL-2 Table for CoronoVac Booster.

	2 doses of CoronaVac + 4 months		3 doses of CoronaVac + 1 month	
	CD4+ IL-2	CD8+IL-2	CD4+ IL-2	CD8+IL-2
1	0.31%	0.17%	0.35%	0%
2	0.29%	0.23%	0.37%	0.23%
3	0.58%	0.64%	0.48%	0.58%

4	0.29%	0.23%	0.37%	0.23%
5	0.44%	0.78%	0.32%	0.34%
6	0.47%	0.29%	0.29%	0.46%